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14. ABSTRACT state the purpose, scope and major findings

The goal of this project was to advance high-altitude medical research by discovering the basic molecular mechanisms of acclimatization and de-acclimatization that protect soldiers from high-altitude illness. This was a large, multi-national collaborative project that will pay dividends for years to come. Eight papers are now published, with two more in review, and four more in preparation. Even though funding for the project has terminated, the science is so compelling that scientists around the world will be working for the next year or two to complete reporting on the basic findings. We have made three major breakthroughs in the AltitudeOmics study: 1) We have identified many hundreds of new mechanisms related to acclimatization, 2) including discovery of epigenetic modifications of key hub genes that may be targets suitable for pharmacologic manipulation to improve performance at high altitude and 3) revealed that acclimatization to hypoxia may yield new information about hypoxia-linked diseases.

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INTRODUCTION:

The goal of this project was to advance high-altitude medical research by discovering the basic molecular
mechanisms of acclimatization and de-acclimatization that protect soldiers from high-altitude illness. We have achieved and surpassed that goal.

BODY:

All major milestones for this project have been accomplished:

- IRB compliance and continuing review have been completed. Continuing review will continue for at least two years while the protocols remain open for analysis.
- Analyses are completed for all subjects at all time points for epigenetics, gene expression, microRNA and metabolomics in blood, and are being undertaken now in add-on studies of muscle function in our subjects.
- All cytokine arrays are done, with follow-up and validation ELISAs completed. Analysis of these responses to hypoxia is underway and will be incorporated as necessary into the gene expression and epigenetics papers.
- Eight papers have been published{Subudhi, 2014 #7347;Amann, 2013 #206;Fan, 2014 #1386;Goodall, 2014 #1673;Roach, 2014 #6740;Ryan, 2014 #6849; Subudhi, 2014 #7348;Subudhi, 2014 #7353}, three in Journal of Applied Physiology, one in Experimental Physiology, one in Acta Scandinavica, one in NeuroReports and two in PLOS ONE. Another two papers are in review at Journal of Applied Physiology. Another two papers ar ein draft form and are included in the Appendix, and six more are in preparation.
- Analyses in blood for nitric oxide, adenosine and hydrogen sulfide are done. Metabolmics for similar blood or red blood cell samples are underway. Once complete these data will be combined into one or more manuscripts.
- The Xia and Blackburn laboratory at the University of Texas at Houston has completed analysis of adenosine, 2,3-BPG, p50 and AMPK levels from our samples, and the Genadkin laboratory in Turku, Finland has completed analysis of ADP, ATP and purigenic receptors. A manuscript with Drs. Eltzschig, Genadkin, Blackburn, and Xia on adenosine in AltitudeOmics is now in draft form and is attached in the draft section of publications. The work on adenosine led to two pending DoD grant applications to exploit this work for rapid translation into solutions to improve soldier performance at high altitude.
- The Lovering laboratory at the University of Oregon, home of our collaborators on AltitudeOmics, have two papers in preparation on AMS and intrapulmonary shunts, and one on gas exchange during AltitudeOmics.

- The Chicco laboratory at Colorado State University is finishing up proteomics assays of skeletal muscle biopsies. The Hansen laboratory at the University of Colorado is currently finishing up metabolomics assays of the same samples. The skeletal muscle biology aspect of the AltitudeOmics study is a complete add-on, paid for entirely by extramural funds. If we can raise enough extra money we will also do transcriptomics and epigenomics on the muscle samples. We expect at least one major paper from this work, perhaps more.

- We are still working on papers integrating the findings from the extensive physiological studies and the OMICS studies. Since no one has done that work before, we are inventing the methods and approaches as we go along. A major breakthrough has been the application of an advanced clustering algorithm called WGCNA to our datasets. This has allowed us to condense the enormous datasets generated by the gene expression and epigenetics studies into a manageable system that can easily be tested for relationships to physiological tests. A first draft of that paper is attached in the draft section of publications.

KEY RESEARCH ACCOMPLISHMENTS:

1. Completed the first ever measurements of oxygen transport, acute mountain sickness, cognitive function and exercise capacity after 7 and 21 days of de-acclimatization. The results suggest near complete retention of acclimatization after 7 days de-acclimatization, and about 70% retention after 21 days. This key finding will be used in the OMICS analyses to help identify factors that occur with acclimatization, and are still present after de-acclimatization.

2. Eight research papers have been completed and published on the physiology of human acclimatization to high altitude. Two papers are in review. Two papers are in draft form, and six additional papers will be completed in the next 12 months. Please see Appendices section for a table showing the "Status of Research Papers" and for a PDF of the published and draft papers, and for a copy of the two grant applications that are a direct outcome of this study.

   Specific accomplishments for each of the eight papers published so far include:

   a. Subudhi AW, Bourdillon N, Bucher J, Davis C, Elliott JE, Eutermoster M, Evero O, Fan JL, Jameson-Van Houten S, Julian CG, Kark J, Kark S, Kayser B, Kern JP, Kim SE, Lathan C, Laurie SS, Lovering AT, Paterson R, Polaner DM, Ryan BJ, Spira JL, Tsao JW, Wachsmuth NB, Roach RC. **AltitudeOmics: The integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent**. PLoS One 2014;9:e92191. {Subudhi, 2014 #7347} This paper provides an overview of the complete AltitudeOmics study and contains novel observations on the process of acclimatization and the retention of the memory of acclimatization on renascent. At 16 days at 5260 m we observed: 1) increases in arterial oxygenation and [Hb] (compared to acute hypoxia: PaO_2 and [Hb] rose while $PaCO_2$ dropped, 2) no AMS; 3) improved cognitive function; and 4) improved exercise performance (all changes $p<0.01$). Upon reascent, we observed retention of arterial oxygenation but not [Hb], protection from AMS,

retention of exercise performance, less retention of cognitive function; and noted that some of these effects lasted for 21 days. Taken together, these findings reveal new information about retention of acclimatization, and can be used as a physiological foundation to explore the molecular mechanisms of acclimatization and its retention.

b. Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC. AltitudeOmics: **On the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans**. J Appl Physiol (1985) 2013;115:634-42. {Amann, 2013 #206} This study of peripheral fatigue showed that acclimatization to severe altitude does not attenuate the substantial impact of hypoxia on the development of peripheral fatigue. In contrast, acclimatization attenuates, but does not eliminate, the exacerbation of central fatigue associated with exercise in severe acute hypoxia.

c. Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT, Roach RC. **AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure.** J Appl Physiol (1985) 2014;116:911-8. {Fan, 2014 #1386} This study of the control of cerebrovascular responses to hypoxia revealed that there was good agreement between steady-state and modified rebreathing estimates of middle cerebral artery blood flow velocity and ventilation responses to carbon dioxide across all three time points ($P < 0.001$, pooled data). Regardless of the method of assessment, altitude acclimatization elevates both the cerebrovascular and ventilatory responsiveness to carbon dioxide. Our data further demonstrate that this enhanced ventilatory carbon dioxide response is partly retained after 7 days at low altitude.

d. Goodall S, Twomey R, Amann M, Ross EZ, Lovering AT, Romer LM, Subudhi AW, Roach RC. **AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude.** Acta Physiol (Oxf) 2014;210:875-88. {Goodall, 2014 #1673} This study of exercise-induced supraspinal fatigue showed that exacerbated supraspinal fatigue after exercise in acute hypoxia is attenuated after 14 days of acclimatization to hypoxia. The reduced development of supraspinal fatigue in chronic hypoxia may have been attributable to increased corticospinal excitability, consequent to an increased cerebral oxygen delivery.

e. Roach EB, Bleiberg J, Lathan CE, Wolpert L, Tsao JW, Roach RC. AltitudeOmics: **Decreased reaction time after high altitude cognitive testing is a sensitive metric of hypoxic impairment.** Neuroreport 2014. {Roach, 2014 #6740} This study of serial reaction time measured before and after a series of other cognitive function tests was a sensitive marker of acclimatization in cognitive function. Specifically, our results suggest that SRT change score (dSRT = SRT1 - SRT2) is a potentially

useful analytical method to enhance the sensitivity of neurocognitive assessment.

f. Ryan BJ, Wachsmuth NB, Schmidt WF, Byrnes WC, Julian CG, Lovering AT, Subudhi AW, Roach RC. **AltitudeOmics: Rapid Hemoglobin Mass Alterations with Early Acclimatization to and De-Acclimatization from 5260 m in Healthy Humans.** PLoS One 2014;9:e108788. {Ryan, 2014 #6849} This paper reports changes in hemoglobin mass during acclimatization. The study showed that that Hbmass increases within 7 days of ascent to 5260 m but that the altitude-induced Hbmass adaptation is lost within 7 days of descent to 1525 m. The rapid time course of these adaptations contrasts with the classical dogma, suggesting the need to further examine mechanisms responsible for Hbmass adaptations in response to severe hypoxia.

g. Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT, Panerai RB, Roach RC. **AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness.** J Appl Physiol (1985) 2014;116:724-9. {Subudhi, 2014 #7348} From this study of cerebral autoregulation (CA) during acclimatization we concluded that alterations in CA are an intrinsic consequence of hypoxia and are not directly related to the occurrence or severity of acute mountain sickness.

h. Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT, Roach RC. **AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery**. Exp Physiol 2014;99:772-81.{Subudhi, 2014 #7347}From this study of cerebral blood flow and oxygenation during acclimatization we concluded that cerebral oxygen delivery (DO2) is well maintained upon acute exposure and acclimatization to hypoxia, particularly in the posterior and inferior regions of the brain associated with vital homeostatic functions. This tight regulation of cerebral DO_2 was achieved through integrated adjustments in local vascular resistances to alter cerebral perfusion during both acute and chronic exposure to hypoxia.

3. Accomplishments to be reported in the adenosine project include the first ever identification of a signaling pathway between blood concentrations of adenosine and 2,3-BPG. 2,3-BPG is a compound that controls how tightly oxygen is bound to hemoglobin. The increase in blood adenosine concentration that occurs in hypoxia, and that rises from acute to chronic hypoxia causes an elevation in 2,3-BPG levels which will aid diffusion of oxygen to the working skeletal muscle. Our collaborators at the University of Texas at Houston have taken this observation and shown in a series of elegant mouse studies that one of the key adenosine receptors is responsible for modulating this response. Thus identifying a target for pharmacologic intervention to boost adenosine levels and perhaps improve performance at high altitude.

4. Combined with the adenosine work, we conducted add-on studies of nitric oxide (NO) and hydrogen sulfide (H2S) in blood during acclimatization. Known as gasotrasmitters,

NO and H2S are potent vasodilators as is adenosine. All three substances are elevated as people become acclimatization. This suggests that peripheral vasodilation is a key aspect of acclimatization, and thus that factors that aid acclimatization likely would aid acclimatization. This is a new avenue of investigation for counteracting the challenge of hypoxia.

5. Skeletal muscle bioenergetics, transcriptomics, epigenomics, proteomics and metabolomics were another first-of-its-kind add-on project that already has shown important new information about changes in skeletal muscle bioenergetics with acclimatization. The completely new metabolism findings from AltitudeOmics suggest that muscle mitochondria respond to hypoxia by increasing their capacity for fatty acid oxidation and improving the coupling efficiency of oxidative phosphorylation without altering the total respiratory capacity of skeletal muscle. The various omics results are coming in as we raise money to fund them, and they promise to reveal the mechanisms at play in this exciting aspect of human adaptation to hypoxia.
6. The transcriptomic and epigenomic data revealed several important findings on this first ever experiment in humans adjusting to hypoxia.
    a. We discovered that thousands of genes are differentially expressed between sea level and 7 days at high altitude, and that many of those genes return to their sea level value of expression by the 16^{th} day. We think of this as processes that trigger acclimatization.
    b. We further discovered that groups of genes behave similarly during acclimatization, and that by using that behavior we can condense millions of genes and their methylation sites into a couple dozen groups of genes that are likely responsible for acclimatization.
    c. We discovered that within these groups of genes, known as modules, that there are key hubs that provide plausible targets for manipulation to alter acclimatization. We already have planned mouse experiments where we will knock out key hub genes to test the hypothesis that response to hypoxia will be improved in the knockout mice that mimic gene expression changes observed in humans.
    d. We also discovered that hypoxia responsive genes behave differently in healthy humans adjusting to hypoxia compared to patients ill with chronic obstructive pulmonary disease (COPD). The healthy humans have a hypoxia-countering response that allows them to improve their oxygen utilization whereas the patients seem unable to mount this counterattack and thus suffer the consequences of low blood oxygen levels. Discussions are underway with COPD researchers to design animal experiments to leverage this new knowledge to develop novel approaches to improving blood oxygen levels in COPD patients.

REPORTABLE OUTCOMES:

1. Completed all regulatory steps to gain approval for this multi-site, multi-nation study. This is an on-going process as we must have human subjects research approval even when no human subject interaction is planned but analysis is on-going. We expect to complete the analysis in the next 24 months at which time we will close all protocols.

2. Safely completed data collection on 23 young healthy student volunteers, and safely transported and cared for them and 40 scientists to/from Bolivia.
3. 8 manuscripts have been published, 2 are under review, 2 more are in draft form, and 6 more are in preparation.
4. Our work has been presented at national and international meetings, including in 2014 a dedicated symposium at the American Physiological Society, a featured presentation at the American Thoracic Society by Dr. Roach, in a poster presentation at the Keystone Hypoxia Symposium, an oral presentation by Dr. Chicco at the International Society for Mountain Medicine, another dedicated symposium at the ICSPP, key presentations by Dr. Roach at the International Society for Systems Biology in Melbourne, Australia and the Third Leh Symposium on CardioPulmonary Adjustments to High Altitude in Leh, Ladakh, India. Abstracts for the above listed presentations are included in the Appendix.
5. We observed that human fully retain acclimatization after 7 days at low altitude, and largely retain acclimatization after 21 days. The mechanisms responsible for this memory of hypoxia are under investigation.
6. Made first ever observations of transcriptomics, epigenomics and metabolomics in humans adjusting to hypoxia. These observations will serve as the basis for many future studies by us and other scientists into the mechanisms that control human responses to hypoxia and how those mechanisms can be manipulated to counteract the harmful effects of low oxygen, whether it is encountered by troops rapidly transported to high altitude or veterans suffering from COPD.

CONCLUSION:

This first ever study of the molecular and cellular mechanisms of human adaptation to hypoxia have already borne significant results, and papers in development will cement the standing of this as one of the major studies in the field of hypoxia research. Already results have been transformed into future grant applications with novel approaches to improve oxygen transport. Also under active development are many new areas of investigation focused on improving human performance in hypoxia. The next decade of work in our lab and also for many of our collaborators will be spent capitalizing on the findings of AltitudeOmics to improve performance and safety of troops exposed to high altitude, and we propose to treat chronic respiratory and cardiovascular diseases where hypoxia is a threat.

REFERENCES

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2. Fan JL, Subudhi AW, Evero O, et al. AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure. J Appl Physiol (1985) 2014;116:911-8.

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5. Ryan BJ, Wachsmuth NB, Schmidt WF, et al. AltitudeOmics: Rapid Hemoglobin Mass Alterations with Early Acclimatization to and De-Acclimatization from 5260 m in Healthy Humans. PLoS One 2014;9:e108788.
6. Subudhi AW, Bourdillon N, Bucher J, et al. AltitudeOmics: The integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent. PLoS One 2014;9:e92191.
7. Subudhi AW, Fan JL, Evero O, et al. AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. J Appl Physiol (1985) 2014;116:724-9.
8. Subudhi AW, Fan JL, Evero O, et al. AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. Exp Physiol 2014;99:772-81.

Appendices

AltitudeOmics: The Integrative Physiology of Human Acclimatization to Hypobaric Hypoxia and Its Retention upon Reascent

Andrew W. Subudhi[1,2], Nicolas Bourdillon[3], Jenna Bucher[4], Christopher Davis[1], Jonathan E. Elliott[4], Morgan Eutermoster[1], Oghenero Evero[1], Jui-Lin Fan[3,5], Sonja Jameson-Van Houten[1], Colleen G. Julian[1], Jonathan Kark[1], Sherri Kark[1], Bengt Kayser[3], Julia P. Kern[4], See Eun Kim[4], Corinna Lathan[6], Steven S. Laurie[4], Andrew T. Lovering[4], Ryan Paterson[1], David M. Polaner[7], Benjamin J. Ryan[8], James L. Spira[9], Jack W. Tsao[10], Nadine B. Wachsmuth[11], Robert C. Roach[1]*

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Abstract

An understanding of human responses to hypoxia is important for the health of millions of people worldwide who visit, live, or work in the hypoxic environment encountered at high altitudes. In spite of dozens of studies over the last 100 years, the basic mechanisms controlling acclimatization to hypoxia remain largely unknown. The AltitudeOmics project aimed to bridge this gap. Our goals were 1) to describe a phenotype for successful acclimatization and assess its retention and 2) use these findings as a foundation for companion mechanistic studies. Our approach was to characterize acclimatization by measuring changes in arterial oxygenation and hemoglobin concentration [Hb], acute mountain sickness (AMS), cognitive function, and exercise performance in 21 subjects as they acclimatized to 5260 m over 16 days. We then focused on the retention of acclimatization by having subjects reascend to 5260 m after either 7 ($n = 14$) or 21 ($n = 7$) days at 1525 m. At 16 days at 5260 m we observed: 1) increases in arterial oxygenation and [Hb] (compared to acute hypoxia: PaO_2 rose 9 ± 4 mmHg to 45 ± 4 while $PaCO_2$ dropped a further 6 ± 3 mmHg to 21 ± 3, and [Hb] rose 1.8 ± 0.7 g/dL to 16 ± 2 g/dL; 2) no AMS; 3) improved cognitive function; and 4) improved exercise performance by $8\pm8\%$ (all changes $p<0.01$). Upon reascent, we observed retention of arterial oxygenation but not [Hb], protection from AMS, retention of exercise performance, less retention of cognitive function; and noted that some of these effects lasted for 21 days. Taken together, these findings reveal new information about retention of acclimatization, and can be used as a physiological foundation to explore the molecular mechanisms of acclimatization and its retention.

Citation: Subudhi AW, Bourdillon N, Bucher J, Davis C, Elliott JE, et al. (2014) AltitudeOmics: The Integrative Physiology of Human Acclimatization to Hypobaric Hypoxia and Its Retention upon Reascent. PLoS ONE 9(3): e92191. doi:10.1371/journal.pone.0092191

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Competing Interests: CL works for AnthroTronix, the developer of the DANA neurocognitve test, which represents a financial competing interest. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

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Introduction

Millions of people live and work in, or travel to, high altitudes, and many of them are able to adjust successfully to the hypoxic environment of very high altitudes (~5000 m), where ambient oxygen pressure is about half the sea level value. Discovery of the mechanisms responsible for human acclimatization to hypoxia could lead to new ways to improve acclimatization and its retention.

The physiology of how humans respond acutely and adapt to hypoxia has been explored extensively over the last century, yet many questions remain about the attributes that best characterize acclimatization [1]. Most would agree that improving arterial oxygenation and exercise performance are central tenets of

acclimatization, and although no studies have focused on the protection from high-altitude illness that occurs with acclimatization, most would also agree such protection is an important aspect of acclimatization. On the other hand, how cognitive function responds during acclimatization is largely unknown, except from anecdotal reports. Intriguing also are suggestions that acclimatization causes functional modifications that persist upon return to high altitude after weeks, or perhaps even months, at sea level, and at a time when all known physiological measures of acclimatization have returned to normal low altitude values [2–4].

AltitudeOmics is a multifaceted research program on acclimatization to high altitude and the retention of acclimatization after return to low altitude. The goals for AltitudeOmics were 1) to describe a phenotype for successful acclimatization and assess its retention—that is—whether adaptive responses persist after descent to low altitude for one to three weeks, and 2) to use these findings as a foundation for companion mechanistic studies of the human transcriptome, epigenome, metabolome, and proteome (OMICS). Our approach was to study lowland volunteers in the field who were taken rapidly to 5260 m, where they acclimatized for 16 days. They then descended to 1525 m for either seven (n = 14) or 21 (n = 7) days, after which they returned quickly to 5260 m and were retested. This report describes the physiology of acclimatization and its retention for four key features of acclimatization: 1) arterial oxygenation and [Hb]; 2) acute mountain sickness (AMS); 3) cognitive function; and 4) exercise performance. Of particular interest was the acclimatization retention displayed upon returning to 5260 m after even three weeks at low altitude. Subsequent reports will explore changes in OMICS responses and will attempt to link those responses to the physiological phenotype of acclimatization and its retention reported here.

Table 1. General Subject Characteristics.

ID	Gender	Age (years)	HT (cm)	WT (kg)	BMI (kg/m^2)
001	M	22	184.2	80.8	23.8
002	M	22	181.6	65.4	19.8
003	F	21	166.4	54.3	19.6
004	M	21	181.6	70.7	21.4
005	F	21	160.0	53.2	20.7
006	M	19	170.2	68.1	23.5
007	M	21	184.2	73.3	21.6
010	F	19	163.8	67.6	25.1
011	F	21	169.5	68.0	23.6
012	M	20	181.6	82.4	24.9
013	M	23	182.9	77.0	23.0
014	M	21	186.7	85.4	24.4
015	F	22	168.3	56.7	20.0
017	F	23	174.0	69.9	23.0
018	M	21	180.3	79.9	24.5
019	F	19	176.5	68.0	21.8
020	F	19	165.7	62.2	22.6
021	M	20	182.9	68.9	20.6
022	M	23	180.3	73.8	22.6
023	M	20	179.1	77.8	24.2
025	F	19	172.1	60.9	20.5
Mean	12M/9F	20.8	175.8	69.7	22.4
SD		1.4	7.9	9.0	1.8

Height (HT); Weight (WT); Body Mass Index (BMI).
doi:10.1371/journal.pone.0092191.t001

Methods

Ethical Approval and Subject Recruitment

The study was performed according to the Declaration of Helsinki. It was approved by the Institutional Review Boards of the Universities of Colorado and Oregon and by the Human Research Protection Office of the U.S. Department of Defense. The subjects were informed about the possible risks and discomforts of participation in the study before giving their written and verbal consent to participate. Physical examinations and the U.S. Army Physical Fitness Test (APFT) (push-ups, sit-ups, and a 3.2-km run) [5] were performed to characterize health and fitness status. Exclusion criteria included: being born at >1500 m; having traveled to altitudes >1000 m in the past three months (including air travel); using prescription medications; smoking; being pregnant or lactating; having a history of serious head injury (loss of consciousness); self or familial history of migraine; known hematologic or cardiovascular abnormality (e.g., sickle cell trait, cardiac arrhythmia); pulmonary function or diffusion capacity for carbon monoxide <90% of predicted; or failure to meet the minimal age/gender standards for the APFT [5]. Seventy-nine subjects completed the screening. Twenty-four healthy, physically active subjects were enrolled. Two subjects dropped out for non-altitude related medical reasons, and one was never healthy at high altitude due to non-altitude related persistent gastrointestinal illness. Thus, 21 subjects (12 males and nine females, average age 20.8 yrs, range 19–23 yrs) constitute the AltitudeOmics group of subjects included in this and subsequent reports (Table 1).

Timeline. Each subject was studied near sea level (SL) (130 m, average $P_B = 749$ mmHg, Figure 1), and over three study periods at Mt Chacaltaya, Bolivia; 5260 m; average $P_B = 406$ mmHg); on the first/second and sixteenth/seventeenth days at 5260 m (ALT1, ALT16), and again upon reascent to 5260 m, after either seven (n = 14) or 21 (n = 7) days at low altitude (POST7 or POST21). Baseline studies at SL, including laboratory (physiologic and OMICS) and field (3.2-km uphill run) tests, were conducted over a two-week period in Eugene, OR, USA. Approximately one month after the SL studies, subjects traveled to Bolivia in pairs on successive days. Upon arrival at El Alto (4050 m) after an overnight flight, subjects immediately descended to Coroico, Bolivia (1525 m; $P_B = 639$ mmHg). Subjects rested for 48 hrs in Coroico to limit the effects of jet lag and were then driven over three hrs to 5260 m. To provide an acute change in inspired PO_2 from 1525 m to 5260 m, subjects breathed supplemental oxygen (2 L/min, nasal cannula or mask) during the drive. On arrival at 5260 m, the first subject immediately began the experimental protocol described below. The second subject rested while continuing to breathe supplemental oxygen for ~ two hrs until the first subject had completed the arterial/venous catheterization and cognitive testing portion of the protocol. Then the second subject began the protocol as described for the first subject. Two subjects were studied per day for ALT1, ALT16, POST7, and POST21. After completing laboratory testing and AMS scoring on ALT1, subjects slept overnight on supplemental oxygen to minimize the risk of developing severe high-altitude illness. The next morning, subjects completed a 3.2-km uphill run (305 m elevation gain) before descending by car to La Paz, Bolivia (3800 m; average $P_B = 487$ mmHg) to continue acclimatizing at a lower altitude over three nights (ALT2-ALT4). On ALT4 subjects

visited 5260 m for four to six hrs. On ALT5, they returned to 5260 m, where they remained for an additional 13 days. On ALT16/17 subjects were tested, as on ALT1/2 prior to descending by car to 1525 m. To test physiological retention of acclimatization after living for seven (n = 14) or 21 (n = 7) days at low altitude (1525 m), subjects returned to 5260 m by car, as they did on ALT1 but this time without supplemental oxygen, and completed the POST7/21 testing (detailed below). After completion of a 3.2-km uphill run on POST7/21, the subjects returned home. Assignment to POST7 or POST21 was determined by each subject based on their desire to stay in the field an extra seven or 21 days. While in Bolivia, subjects were housed and fed as a group. Meals and snacks were kept similar to the subjects' typical *ad libitum* diet. Subjects were instructed to ingest at least three liters of water each day and to remain physically active.

Experimental Protocol. Testing progressed in the following general order: 1) radial artery and antecubital vein catheterization; 2) 30-min supine rest, followed by cognitive function testing; 3) measurement of resting (seated) arterial blood gases and hemoglobin concentration, and blood draw for OMICS samples; 4) cycle ergometry exercise testing; 5) AMS symptom scoring; and, on a separate day, 6) a 3.2-km uphill run. In addition to the studies presented here, within the framework of AltitudeOmics and reported separately, we also assessed cerebral blood flow[6] and cerebral autoregulation [7]; chemical control of breathing [8]; total hemoglobin mass and blood volume compartments; peripherally [9] and centrally [10] derived measures of exercise-induced fatigue; blood flow through intracardiac shunt (patent foramen ovale) and intrapulmonary arteriovenous anastomoses; and OMICS responses (transcriptomics, epigenomics, metabolomics, and proteomics).

Procedures

Anthropometry. Height (cm) was measured at SL only. Body mass (kg) was recorded at SL, ALT1, ALT16, and POST7/21 using the same scale (Seca 770, Hanover, MD, USA), with the subject wearing light underwear and no shoes.

Arterial Blood Gases and Hemoglobin

Under local anesthesia (2% lidocaine) a 20–22 G radial artery catheter (Models RA-04122/RA-04020 Arrow International, Reading, PA, USA) was placed for the duration of experiments conducted at SL, ALT1/16, and POST7/21. Arterial blood samples were drawn anaerobically and immediately analyzed in duplicate for PaO_2, $PaCO_2$, pH (Siemens RAPIDLab 248, Erlangen, Germany), [Hb] and SaO_2 (Radiometer OSM3, Copenhagen, Denmark). Core temperature was measured using an ingestible temperature-sensing pill (CorTemp HQInc, Palmetto, FL, USA)[11,12]. Blood gases were corrected for core temperature [11,12]. CaO_2 (mL/dL) was calculated as: $CaO_2 = 1.39 * [Hb] * (SaO_2/100) + (PaO_2 * 0.003)$. The Hill equation was used to calculate P50 [13]. Resting arterial blood samples were taken following 10 min of seated rest at SL, ALT1, ALT16, and POST7/21.

Acute Mountain Sickness

The severity of AMS symptoms was assessed using the Lake Louise Questionnaire (LLQ), which includes a self-reported assessment of AMS symptoms (headache, fatigue, gastrointestinal discomfort, and dizziness) and the shortened Environmental Symptom Questionnaire (AMS-C). Total LLQ scores that included headache and were ≥3 or ≥6 (out of a possible total of 12) were diagnostic of moderate or severe AMS, respectively. Quality of sleep was not included in the total LLQ score because nights prior to ALT1 and POST7/21 were spent at low altitude. Recently, in our laboratory, we have published LLQ without using the sleep question, with no change in sensitivity in identifying AMS [14,15]. AMS-C is a self-reported 11-question inventory from which a score ≥ 0.7 is considered indicative of AMS [16]. AMS symptoms were assessed at SL, ALT1 (in the evening,

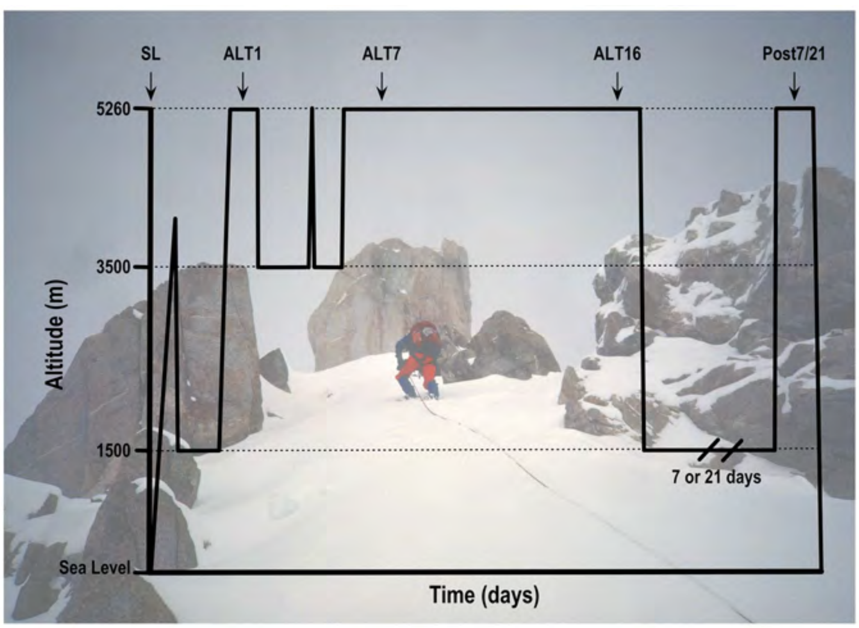

Figure 1. Timeline for AltitudeOmics Studies. Each subject completed this study timeline, with n = 14 staying at low altitude for POST7 and n = 7 staying at low altitude for POST21. Subjects flew from the USA to Bolivia aboard commercial aircraft with no recording of barometric pressure during the flight; the profile for travel in the figure is therefore approximate.
doi:10.1371/journal.pone.0092191.g001

10–12 hrs after arrival), ALT16, and POST7/21 (time-matched to ALT1).

Cognitive Function

The Defense Automated Neurobehavioral Assessment (DANA) was used to assess neurocognitive function. DANA is a neurocognitive assessment tool that includes a library of open-source, standardized, cognitive and psychological assessments [17]. Using a handheld computer, the following nine cognitive function tests were administered: 1) Simple Reaction Time-1 (measured at the beginning of neurocognitive testing to gain an understanding of pure visual-motor response); 2) Simple Reaction Time-2, repeated at the end of neurocognitive testing to assess diminished reserve of cognitive effort on reaction time; 3) Procedural Reaction Time, a measure of choice reaction time and accuracy; 4) Go-No-Go, a measure of speed, accuracy and impulsivity; 5) Code Substitution—Simultaneous, a measure of visual scanning and attention, learning, and immediate recall of digit-symbol pairings; 6) Code Substitution—Delayed Recall, a measure of short-term memory for digit-symbol pairings; 7) Spatial Discrimination, a measure of visuospatial analytic ability; 8) Match to Sample, an assessment of attention and memory for visuospatial discrimination; and 9) Sternberg's Memory Search, a measure of working memory for letters. Neurocognitive tests were administered before and after the expedition at SL and once each at ALT1, ALT16 and POST7/21. Repeat cognitive function tests at SL were similar ($p>0.5$) and thus were combined to give one SL score for comparison to changes in cognitive function at 5260 m. Mean throughput, a measure of mental efficiency, is calculated as the mean number of correct responses for each test made within one min [18] and is the outcome variable reported for all cognitive function variables.

Exercise

Laboratory exercise testing. Incremental exercise tests to maximal exertion on an electrically-braked cycle ergometer (Velotron Elite, Racermate, Seattle, WA, USA) were used to assess peak aerobic power. Subjects completed three-min stages at 70, 100, 130 and 160 Watts, followed by 15 Watts/min increments until they could no longer maintain pedaling at > 50 rpm. Peak aerobic power (Watts) was calculated as: work rate of last stage completed + [(work rate increment) * (time into final stage/duration of stage in seconds)] [19]. Exercise tests were performed at SL, ALT1, and ALT16, but not at POST7/21 due to logistical issues.

Field exercise testing. Subjects completed a timed 3.2-km uphill run as fast as possible, on unpaved roads, with an identical elevation gain of 305 m. Tests were performed at SL at least 48 hrs before the laboratory tests and in the morning after an overnight stay on ALT1, ALT16 and POST7/21. Performance was expressed as mean running speed in m/s.

Data Analysis

As expected, preliminary analyses revealed higher CaO_2 for males as a result of higher [Hb], across the study ($p<0.01$ vs. females); however, since the sex vs. time interaction was not significant ($p>0.05$) male and female data were pooled for all subsequent analyses. For physiological variables, paired t-tests, with Bonferroni correction for multiple testing, were completed for comparisons among time points. LLQ, AMS-C scores and cognitive function tests were evaluated by the Wilcoxon signed rank test. The Spearman rank order and Pearson product moment correlations were run to evaluate associations between changes in arterial blood gases and [Hb] and changes in AMS symptoms, cognitive function, and physical performance across time. Due to transportation delays and the technical challenges inherent to field studies, not all procedures were completed on all subjects at Mt. Chacaltaya (see Tables S1, S2, S3, S4, S5 for respective sample sizes). Overall, most subjects completed most tests, with 88% of arterial blood gas and hematology measurements, 100% completion of AMS and cognitive function tests, and 95% for the 3.2-km uphill run. For all parametric statistical comparisons, $p<0.01$ (Bonferroni correction of 0.05/5) was considered significant, with $p<0.01$ for Wilcoxon signed rank test results considered significant. Individual data for all responses reported here are presented in Tables S1, S2, S3, S4, S5. Data in the text are presented as means ± standard deviation.

Results

Anthropometry

Height and body mass at SL are presented in Table 1. Body mass was unchanged from SL to ALT1 (p = NS), then dropped by 2.6 ± 1.6 kg ($p<0.01$) from ALT1 to ALT16; it showed no significant change thereafter (Table S1).

Arterial Blood Gases and Hemoglobin

PaO_2, $PaCO_2$, SaO_2, and CaO_2 were reduced with acute exposure to 5260 m (SL to ALT1, $p<0.01$; Figure 2, panels A–C, Table S2), while pH and P50 increased ($p<0.01$, Figure 2, panels D and E) and [Hb] was unchanged (p = NS, Figure 2, panel F). PaO_2, SaO_2, CaO_2, P50, and [Hb] all increased from ALT1 to ALT16, while $PaCO_2$ continued to fall ($p<0.01$, all comparisons) and pH was unchanged (p = NS; Figure 2). SaO_2 at POST7 was maintained at ALT16 levels. In contrast, PaO_2, CaO_2, P50, and [Hb] at POST7 decreased from ALT16 ($p<0.01$) and approached ALT1 values. $PaCO_2$ rose at POST7 from ALT16 values and was significantly different from both ALT1 and ALT16 ($p<0.01$). Since subjects studied at POST21 had incomplete arterial blood gas data at all time points but SL; those data are qualitatively discussed, but data in the text and figures are at all time points for the POST7 group only. The pattern of change from ALT16 to POST21 was similar to that seen from ALT16 to POST7 for PaO_2, $PaCO_2$, SaO_2, CaO_2, pH, and [Hb], suggesting possible retention of acclimatized values for SaO_2, but less so for PaO_2, $PaCO_2$, CaO_2, P50, pH, and [Hb].

Acute Mountain Sickness

LLQ and AMS-C were highly correlated ($R^2 = 0.72$, $p<0.001$) and identified the same subjects as AMS positive at ALT1; for brevity, only the LLQ score is discussed (see Table S3). Eighty-one percent (17/21) of subjects had AMS (LLQ≥3; $p<0.01$ vs. SL) on the evening of their first night at 5260 m; of those with AMS nearly half had severe AMS (LLQ≥6; $p<0.01$vs. SL; Figure 3A). AMS completely resolved in all subjects as acclimatization progressed from ALT1 to ALT16. Upon reascent at POST7 subjects remained free from AMS. On POST21, 3/7 of subjects again developed AMS scores≥3 (p = NS vs. ALT16), but none reported severe AMS. Nobody exhibited HAPE or HACE.

Cognitive Function

Repeat tests at sea level pre-post expedition showed no major differences between individuals or group values ($p>0.5$) and were thus averaged to provide a more robust SL value (Table S4a–c). Five of nine neurocognitive tests showed marked decrements from SL to ALT1 (Simple Reaction Time-1, Simple Reaction Time-2, Code Substitution—Simultaneous, Match to Sample and Procedural Reaction Time, $p<0.01$, Figure 4); no change from SL to ALT1 was seen for Code Substitution—Delayed Recall, Spatial Discrimination, Go-No-Go, and Memory Search ($p>0.05$) (Table

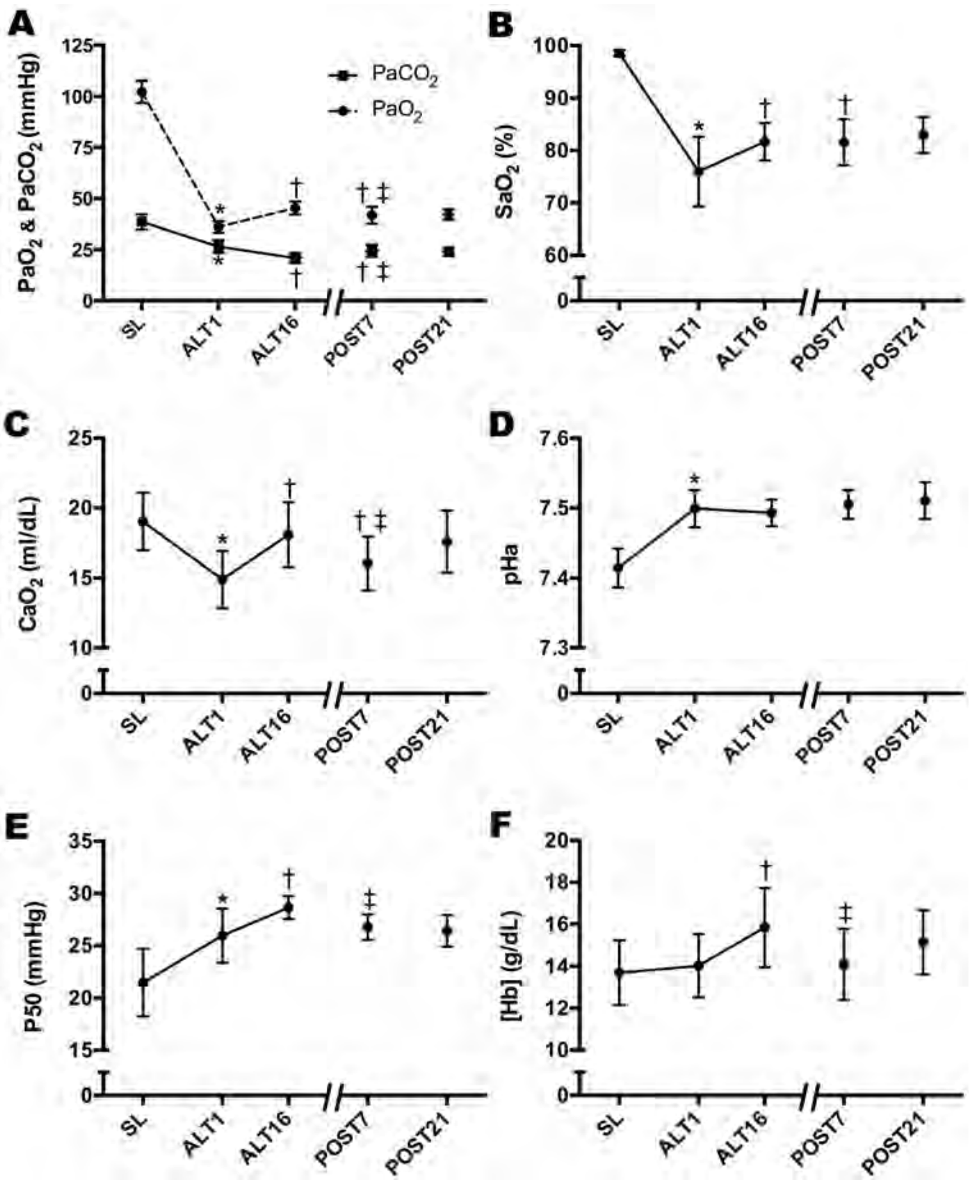

Figure 2. Arterial Blood Gases and [Hb] During Acclimatization and Upon Reascent. Resting indices of ventilatory and hematological acclimatization at SL, ALT1, ALT16, and POST7/21 demonstrating acclimatization after 16 days at a constant altitude and the degree of retention in these variables. *Significantly different vs. SL ($p<0.01$); † significantly different than ALT1 ($p<0.01$); ‡significantly different than ALT16 ($p<0.01$).
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S4a–c). Subsequent analyses focused on the five tests that showed a change with acute hypoxia. Performance improved on Simple Reaction Time-1, Simple Reaction Time-2, Code Substitution—Simultaneous, Match to Sample, and Procedural Reaction Time as acclimatization progressed from ALT1 to ALT16 ($p<0.01$, Figure 4). At POST7, Code Substitution—Simultaneous and Match to Sample showed retention of acclimatization compared to ALT16 ($p<0.01$, Figure 4, panels C and D), with loss of acclimatization evident for Simple Reaction Time-2, Procedural Reaction Time ($p<0.01$, Figure 4, panel B and E), and a trend to loss of acclimatization noted for Simple Reaction Time-1 ($p>0.01<0.05$, Figure 4, panel A). No cognitive function tests showed retention of acclimatization at POST21.

Exercise

Laboratory exercise testing. Peak oxygen uptake at SL was 3.4±0.8 l/min and fell by 29±11% to 2.3±0.6 l/min at ALT1 ($p<0.01$), with no change observed from ALT1 to ALT16 (p = NS) (See Table S5). Peak power output at SL was 265±57 W; it fell by 34±7% to 171±40 W at ALT1 ($p<0.01$), and like peak oxygen uptake, it did not improve with acclimatization. Changes in resting arterial oxygenation and [Hb] from SL to ALT1 to ALT16 were not correlated with peak oxygen uptake (p = NS).

Field exercise testing. Running speed was 44±5% slower at ALT1 compared to SL ($p<0.01$; Figure 5). Running speed improved 8±8% from ALT1 to ALT16 ($p<0.01$) and was maintained at POST7 (p = NS). Subjects maintained acclimatized (ALT16) running speed at POST7 despite 13% lower resting [Hb] and CaO_2. After 21 days at low altitude, running speed tended to be slower than at ALT16 ($p = 0.06$) and was not significantly different

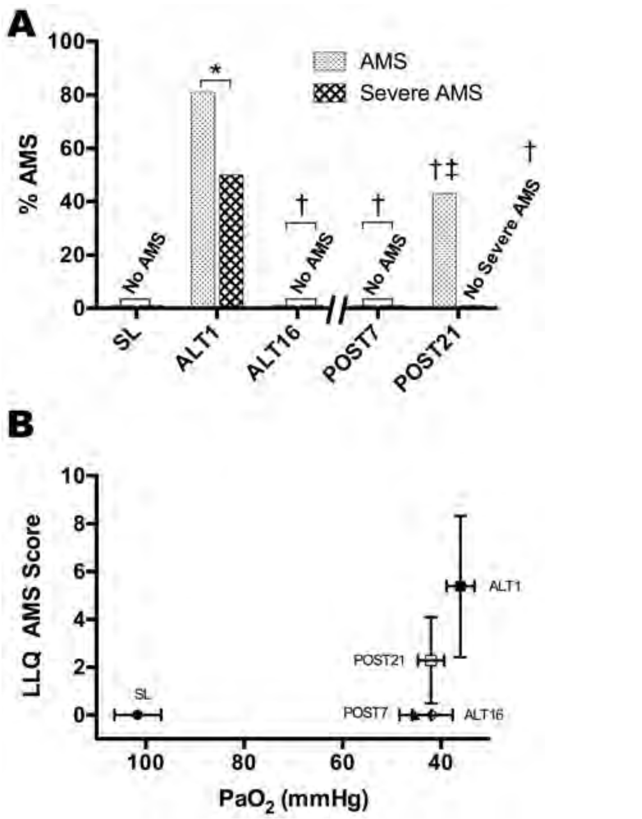

Figure 3. Development of Acute Mountain Sickness, Its Resolution with Acclimatization And Prevention Upon Reascent. Percentage of subjects reporting moderate to severe AMS based on LLQ scores of ≥3, or ≥6, respectively. (A) Symptoms of AMS at ALT1 were alleviated at ALT16 and were largely absent with reascent on POST7/21. (B) Mean PaO_2 and median LL AMS scores reveal no relationship of hypoxemia to AMS. *Significantly different than SL ($p<0.01$); †significantly different than ALT1 ($p<0.01$); ‡significantly different than ALT16 ($p<0.01$).
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from ALT1 (p = NS), suggesting a partial loss of acclimatization in running speed by POST21.

Relationship of AMS, Cognitive Function and Exercise Performance to Arterial Oxygenation and [Hb]

During acclimatization AMS, cognitive function, and exercise performance improved, and for AMS and exercise those improvements were retained upon reascent, with only some tests of cognitive function showing retention of acclimatization. The changes that occurred during acclimatization and upon reascent in PaO_2, $PaCO_2$, SaO_2, CaO_2, P50, pH, and [Hb] were not related on an individual (all correlations $r<0.5$) or group basis (all comparisons $p>0.1$) to AMS, cognitive function, or exercise responses. However, the pattern of change with acclimatization in PaO_2, $PaCO_2$, SaO_2, CaO_2, P50, pH and [Hb] matches the pattern of change for AMS, cognitive function, and exercise performance, suggesting an underlying but complex relationship between oxygenation and other aspects of acclimatization.

Discussion

In this paper, we have presented four aspects of altitude acclimatization through a 16-day initial exposure to 5260 m, and upon reascent to the same altitude after either seven or 21 days at low altitude. We found, as have others before us [20–30], elevated arterial oxygenation and [Hb], resolution of symptoms of acute mountain sickness and increased exercise performance after 16 days residence at 5260 m. We also report improvements in measures of cognitive performance that we believe represent a novel and important additional indicator of acclimatization. Most intriguing was finding that after descending to low altitude for one or three weeks, physiological evidence of acclimatization persisted upon returning to 5260 m, as manifest by less AMS, retention of improved exercise performance, and to some extent cognitive performance.

Physiology of Acclimatization

The elevations in arterial oxygenation and [Hb] from ALT1 to ALT16 were similar to those measured in individuals acclimatized for at least 10 days at altitudes ranging from 3800 m to 5260 m [20,26,29,30]. For example, Lundby et al. reported that [Hb] and CaO_2 increased markedly from SL to two weeks at 4100 m, but did not rise further at eight weeks [26]. While similar data do not exist for the rise in PaO_2 and fall in $PaCO_2$ with ventilatory acclimatization at two and eight weeks at a fixed high altitude, Wagner et al. reported after nine weeks at 5260 m a PaO_2 of 50 ± 1 mmHg and a $PaCO_2$ of 21 ± 0.9, values similar to PaO_2 (45 ± 3) and $PaCO_2$ (21 ± 3) in the present study after 16 days at 5260 m [30]. Thus, it seems that≥14 days at 4000 m to 5000 m results in significant acclimatization, and that this duration of exposure can be effective to test acclimatization and its subsequent retention [30].

Sixteen days of acclimatization at 5260 m was effective in reducing the incidence of AMS from 81% in our subjects upon acute exposure to 0% at ALT16, a finding consistent with existing literature [23,24,28]. These findings suggest a new experimental approach to unraveling the pathophysiology of AMS. To our knowledge, no pathophysiological studies of AMS have taken advantage of the complete protection from AMS conferred by acclimatization by comparing individuals upon acute ascent to when they are acclimatized, or upon reascent when presumably the factors that protect from AMS will stand out from other factors that are epiphenomena to the acclimatization process but not key to AMS prevention.

This is the first report of complete recovery of cognitive function to sea level values after acclimatization to high altitude, supporting the idea that cognitive function is an important outcome of acclimatization. DANA tests have negligible practice effects (other than spatial discrimination, which asymptotes after the second administration) [17]. This was evident in the current study, as no significant differences were detected between DANA measures on pre- and post-expedition SL tests. We found that the five tests showing impairment in acute hypoxia all returned to SL values by ALT16 ($p<0.01$, Figure 4). Barcroft et al. reported anecdotal impairment in cognitive function during acclimatization, but lacked any quantitative evidence [31]. Other studies have reported effects on cognitive function in acute hypoxia [32–36] during experiments and expeditions where the barometric pressure and environmental conditions were different at each testing point, such as occurs during a climbing expedition [37–39], and one has speculated about the recovery of cognitive function with acclimatization [40]. However, none of those studies have shown, as in the present study, that when subjects are studied at the same altitude over the course of acclimatization that cognitive function improves to sea level values. DANA tests speed and accuracy in measures that assess attention, simple discrimination, and immediate and incidental memory. Although these measures offer an indication of working memory, they do not assess complex problem-solving and

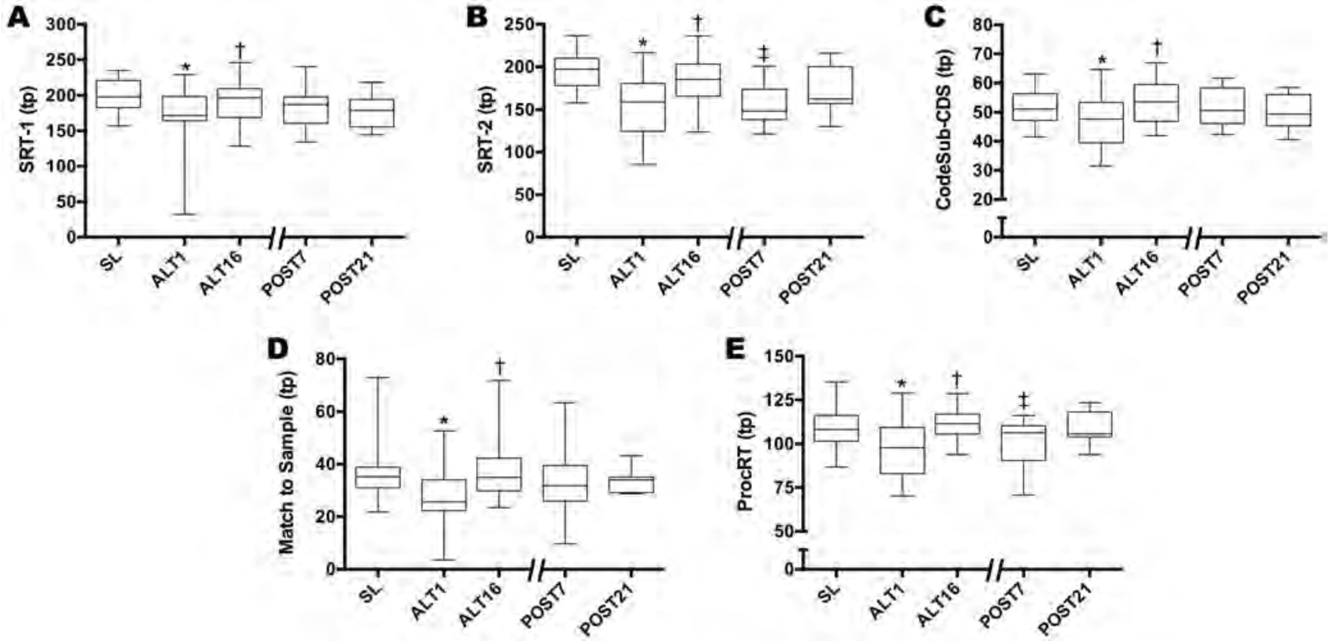

Figure 4. Neurocognitive Function During Acclimatization and Upon Reascent. Five tests of cognitive function revealed marked decrements in performance from SL to ALT1, and improvement back to sea level values by ALT16. Code Substitution—Simultaneous and Match to Sample retained levels found at ALT16 on POST7, while Simple Reaction Time-1, Simple Reaction Time-2, and Procedural Reaction Time essentially reflected a loss of during acclimatization upon reascent at POST7. None of the cognitive function tests showed any retention of acclimatization at POST21. (tp = throughput = mean number of correct responses made within one min). *Significantly different than SL ($p<0.01$); †significantly different vs. ALT1 ($p<0.01$); ‡significantly different vs. ALT16 ($p<0.01$).
doi:10.1371/journal.pone.0092191.g004

decision-making aspects of executive functioning, which may be especially relevant for people working at high altitudes. Understanding the mechanism for the marked resolution of the initial decrement in cognitive performance that occurs in acute hypoxia has potential impact [41] for anyone visiting, living, or working at high altitudes where impaired cognitive dysfunction is a major challenge [37,38,42].

Our findings for submaximal exercise performance are consistent with other reports showing improvements during acclimatization [22,25,27,43] with no change in peak oxygen consumption [2,22,26,44–51]. However, in retrospect, we question the practical relevance of these all-out efforts, as most work or recreational activities at high altitude are not performed to exhaustion or as fast as possible. For example, mountaineers try to preserve energy to sustain efforts across multiple days and might actually put themselves at risk of serious harm, or death, if they truly reached the point of exhaustion. Their ability to cover more ground faster while preserving a functional reserve is a hallmark of acclimatization supported by anecdotal accounts [52,53]. To the best of our knowledge, only one study before the present report has objectively measured this type of submaximal performance [43]. The physiology behind the improvement in sustained, self-regulated submaximal performance at altitude remains unexplored [2,22,26,43–52].

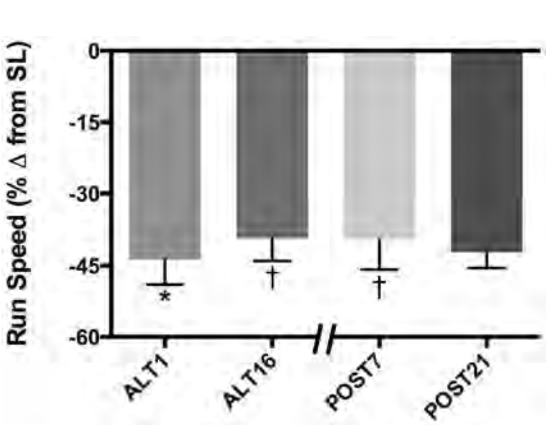

Figure 5. Field Exercise Testing During Acclimatization and Upon Reascent. Uphill running speed plotted as percent change from sea level improved from ALT1 to ALT16 and was retained at POST7, with a trend to retention at POST21. *Significantly different vs. SL ($p<0.01$); †significantly different vs. ALT1 ($p<0.01$).
doi:10.1371/journal.pone.0092191.g005

Physiological Retention of Acclimatization: Arterial Blood Gases and Hemoglobin

At POST7/21, PaO_2 and $PaCO_2$ values ranged between ALT16 and ALT1 values, indicating partial retention of ventilatory acclimatization. In contrast, SaO_2 and pH remained near ALT16 acclimatized levels on POST7/21. We calculated a decreased P50 from ALT16 to POST7/21, suggesting a left shift in the oxyhemoglobin dissociation curve upon reascent as a possible explanation for the retention of acclimatized values for SaO_2 at POST7/21 [2]. These findings are compatible with one previous study showing partial retention of ventilatory acclimatization using noninvasive indices of oxygenation and end tidal CO_2 levels after eight days at low altitude [2,54]. The drop in [Hb] from ALT16 to POST7/21 may be due to selective destruction of the youngest circulating red cells (neocytolysis) upon return to low altitude [55–58], or potentially an increase in plasma volume [59].

Physiological Retention of Acclimatization: Acute Mountain Sickness

Our findings on AMS upon reascent extend the work of others conducted at lower altitudes in demonstrating that previous altitude acclimatization confers some protection from AMS [3,4,60]. The marked efficacy of acclimatization to prevent severe AMS is underscored by comparison to results from clinical trials where acetazolamide only reduced the risk of severe AMS by 44% [61], compared to 100% for acclimatization in our study. Exactly how acclimatization prevents AMS and other high-altitude illnesses upon reascent is unclear.

AMS is clearly triggered by hypoxemia, but once the processes that cause AMS are initiated, the relationship with PaO_2, SaO_2, and CaO_2 is less clear. This is reflected in Figure 3B where AMS scores are highest when PaO_2 is lowest at ALT1, but when at POST7 and ALT16, when PaO_2 levels are only a few mmHg higher than ALT1 values, AMS is absent. Additionally, at POST7, when AMS is absent in all 14 subjects, CaO_2 levels are much lower than at ALT16, suggesting a limited role for CaO_2 in the protection from AMS observed upon reascent. One explanation may be that the absolute value of PaO_2, SaO_2, or CaO_2 is not the critical factor, but rather that acute hypoxia sets in motion the physiological alterations leading to AMS. In other words, perhaps an individual threshold exists that triggers AMS when crossed [62]. Unraveling how this occurs may lead to advances in the understanding of the pathophysiology of high-altitude illnesses.

Physiological Retention of Acclimatization: Cognitive Function

Cognitive function stands out as a key feature of acclimatization to hypoxia that is not completely retained at acclimatized levels upon reascent. The tests that showed retention of acclimatization at POST7 (Code Substitution—Simultaneous and Match to Sample) commonly reflect changes in short-term memory. The tests of reaction time (Simple Reaction Time-1, Simple Reaction Time-2, Procedural Reaction Time) essentially returned to ALT1 values by POST7, indicating a loss of the improvement in reaction time seen with acclimatization. Short-term memory and reaction time appear to represent distinct processes that respond differently to the changes in arterial blood gases and [Hb] from ALT16 to POST7. Understanding the mechanisms responsible for acclimatization retention or its loss could lead to new insights into the links between brain oxygenation and cognitive function for persons at high altitudes.

Physiological Retention of Acclimatization: Exercise

The retention of exercise performance for at least seven days, with partial retention after 21 days spent at low altitude, has important implications for everyone living, visiting, or working at high altitudes. At POST7, and to a lesser extent at POST21, subjects essentially matched their acclimatized running performance. This is the first report of retention of acclimatized exercise performance upon reascent after de-acclimatizing at low altitude. As far as we know, only one other study attempted to measure retention of acclimatized endurance exercise performance [2], but that study showed no improvement in endurance exercise performance with acclimatization, likely due to a small sample size ($n = 6$), thus rendering testing of retention impossible. As noted above, all studies [22,25,27,43] but one [2] have shown improvement in submaximal endurance capacity with acclimatization. The retention of exercise performance shown at POST7 occurred despite significant reductions in resting [Hb] and CaO_2. These findings are contrary to those reporting a direct positive effect of CaO_2 on exercise performance at lower altitudes [25,63], but agree with those reporting little effect of CaO_2 on exercise performance at higher altitudes (>3500 m) [64–66]. If the improvement of exercise performance with acclimatization and its retention upon reascent is not directly related to CaO_2, then other factors must be at play. One possibility is that mechanisms other than oxygen delivery could boost oxygen transport and thus exercise performance during acclimatization and upon reascent, such as elevated circulating blood levels of vasodilatory substances (e.g., nitric oxide [67] or adenosine [68]) or other, as yet unknown, processes. Discovering the mechanisms responsible for improving exercise performance with acclimatization and its retention after acclimatization has potential relevance to exercise tolerance in anyone exposed to hypoxia.

Physiological Mechanisms Explaining Acclimatization and its Retention

Acclimatization transforms a lowlander into someone who is protected from high-altitude illness, has improved cognitive function, and has better exercise performance at 5260 m. In the present study, acclimatization-induced improvements in AMS symptoms, cognitive function and exercise performance appear to follow the time course of ventilatory and hematological acclimatization. But after extensive analysis, no case was found where the degree of improvement in AMS symptoms, cognitive function, and exercise performance was significantly directly correlated to measured indices of arterial oxygenation and [Hb]. Further, arterial oxygenation and [Hb] were poorly correlated with the benefits of acclimatization that persisted upon reascent. Though not well known, Luft et al. reported on the retention of acclimatization based on studies conducted in hypobaric chambers on climbers returning from Nanga Parbat in 1938 [69]. The measurement of retention was tolerance to very high altitudes (>8000 m) measured, in part, by deterioration of handwriting. They noted that neither the hemoglobin concentration nor the erythrocyte count were responsible for the persistence of acclimatization. While we acknowledge the inherent limitations of correlational analyses, the disconnection between ventilatory and hematological acclimatization and physiological function suggests that additional mechanisms are involved in acclimatization and its retention. These might include physiological responses that we did not measure, or molecular and cellular responses in a specific tissue such as brain that cannot be easily measured in humans. In subsequent reports we will pursue a linkage between the OMICS responses and the physiological adjustments described here to explore the mechanisms underlying acclimatization to high altitude and its retention.

Limitations

Several limitations in the study design and execution should be considered. This study was completed in the field, in a foreign country, and with many uncontrolled variables. The rationale for this approach over a trial in a hypobaric environment where many more variables could be controlled was that such a large study could not be completed for a reasonable cost and in a reasonable time-frame in a hypobaric chamber. Operation Everest II studied six-to-eight subjects during a 40-day simulated ascent of Mt Everest. Though many of the time points from Operation Everest II had data from only four to six subjects, many important observations were made from these experiments [29,44,70–73]. But to have sufficient statistical power to combine the OMICS and physiological studies, much larger sample sizes are needed. As far as the authors know, there is one hypobaric chamber in the world large enough to accommodate 21 subjects at a time, located in

Glasgow, Scotland. While we acknowledge the field design as a limitation, we believe this study makes an important contribution to understanding acclimatization that can point to future studies with smaller samples and more focused experimental questions in controlled hypobaric chamber conditions.

This study was limited to 16 days of acclimatization. While this was sufficient time to see marked changes in the variables measured, it is unclear if longer exposure would have resulted in further improvements in acclimatization or better retention of acclimatization upon reascent. Also, due to logistical and financial constraints and to avoid areas of high malarial risk, subjects did not descend all the way to sea level between exposures. However, this may not be a major concern, since our results are consistent with other studies reporting protection from AMS after acclimatization [3,4,60]. Only Lyons et al. [3] reported data from a controlled study of acclimatizing individuals; others used epidemiological observations suggesting AMS protection from acclimatization [4,60]. Also, we made no measurements at low altitude prior to reascent, so a question remains as to how much of the reascent responses were present at low altitude such as hyperventilation, resulting in low $PaCO_2$, versus how much was nascent at low altitude but was rapidly triggered on re-ascent.

An additional concern is that subjects may have de-trained over the 16 days at high altitude, since they were unable to completely maintain their regular exercise regimen. When back at low altitude, subjects resumed their habitual levels of physical activity, potentially restoring some fitness and confounding our measures of exercise performance. Also, changes in total and lean body mass across the study may have affected physical performance [74], but since changes in body composition and training status are inherent to life at high altitude, we feel our results have strong practical relevance.

Finally, the AltitudeOmics project encompasses an extensive suite of physiological and OMICS measurements, and, in its entirety, produced more than 60 million individual data points. Consequently, the data has been partitioned into discrete papers with the ultimate goal of a series of publications that are individually robust and as comprehensive as possible. The physiological parameters included in this paper have historically been used to describe acclimatization, and thus were deemed appropriate as a bridge between past studies and the novel discoveries from AltitudeOmics. Further publications will explore the process of acclimatization by utilizing additional OMICS and physiological data whose inclusion excessively widened the scope of the current paper.

Conclusion

In this study of acclimatization to a very high altitude, we found improvements in key variables after 16 days that describe an acclimatized phenotype by partial acclimatization for arterial oxygenation and [Hb], absence of high-altitude illness, improved cognition and exercise performance. Another intriguing observation is that after descending to low altitude for one or three weeks, evidence of acclimatization persists, as manifested by an acclimatized value for SaO_2, much less severe AMS, maintained exercise performance, and to a lesser extent retention of acclimatized cognitive performance. During the time at low altitude, many of the changes reflecting ventilatory and hematologic adaptation returned to or toward the unacclimatized state at the time reascent measurements were made. In conclusion, this study identifies a phenotype of successful human acclimatization to hypoxia, identifies novel aspects of the retention of the acclimatized phenotype after time at low altitude, and will serve as a foundation for comparing the phenotype of acclimatization with potential mechanistic mediators of acclimatization derived from companion studies of the human transcriptome, epigenome, metabolome, and proteome.

Supporting Information

Table S1 Body Composition. Individual body weight data at SL, ALT1, ALT16, POST7 and POST21 and body fat and lean body mass at SL, ALT1, and ALT16.
(PDF)

Table S2 Resting Arterial Blood Gases and Hemoglobin Concentration. Individual resting arterial blood gases and [Hb] data at SL, ALT1, ALT16, POST7 and POST21.
(PDF)

Table S3 Acute Mountain Sickness Scores for Lake Louise (LLQ) and Environmental Symptom (AMS-C) Questionnaires. Individual AMS symptom scores and the composite LL and AMS-C scores at SL, ALT1, ALT16, POST7 and POST21.
(PDF)

Table S4 a. Cognitive Function Tests. Individual cognitive function test scores for Simple Reaction Time-1, Simple Reaction Time-2, Code Substitution—Simultaneous, and Code Substitution—Delayed Recall at SL, ALT1, ALT16, POST7 and POST21. b. Cognitive Function Tests. Individual cognitive function test scores for Spatial Discrimination, Go-No-Go, Sternberg's Memory Search, and Matching to Sample at SL, ALT1, ALT16, POST7 and POST21. c. Cognitive Function Tests. Individual cognitive function test score for Procedural Reaction Time at SL, ALT1, ALT16, POST7 and POST21.
(PDF)

Table S5 Peak Power Output and Submaximal Exercise Performance. Individual maximal exercise performance and 5-km time to completion data at SL, ALT1, and ALT16 and field exercise testing results at SL, ALT1, ALT16, POST7 and POST21.
(PDF)

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Author Contributions

Conceived and designed the experiments: NB JEE OE JLF SJVH CGJ BK JPK SSL ATL AWS RCR. Performed the experiments: NB JB CD JEE ME OE JLF SJVH JK SK BK JPK SEK CL SSL ATL RP DMP BJR JLS AWS JWT NBW RCR. Analyzed the data: NB JEE OE JLF SJVH BK JPK CL SSL ATL BJR JLS AWS NBW RCR. Wrote the paper: NB OE JLF BK AWS RCR. Revised the Manuscript: NB JB CD JEE ME OE JLF SJVH CGJ JK SK BK JPK SEK CL SSL ATL RP DMP BJR JLS AWS JWT NBW RCR.

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AltitudeOmics: cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness

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[1]*University of Colorado Altitude Research Center, Department of Emergency Medicine, Anschutz Medical Campus, Aurora, Colorado;* [2]*University of Colorado Colorado Springs, Department of Biology, Colorado Springs, Colorado;* [3]*University of Lausanne, Institute of Sports Sciences, Lausanne, Switzerland;* [4]*University of Geneva, Lemanic Doctoral School of Neuroscience, Geneva, Switzerland;* [5]*University of Oregon, Department of Human Physiology, Eugene, Oregon; and* [6]*University of Leicester, Leicester Royal Infirmary, Department of Cardiovascular Sciences, Leicester, United Kingdom*

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Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT, Panerai RB, Roach RC. AltitudeOmics: cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. *J Appl Physiol* 116: 724–729, 2014. First published December 26, 2013; doi:10.1152/japplphysiol.00880.2013.—Cerebral autoregulation (CA) acts to maintain brain blood flow despite fluctuations in perfusion pressure. Acute hypoxia is thought to impair CA, but it is unclear if CA is affected by acclimatization or related to the development of acute mountain sickness (AMS). We assessed changes in CA using transfer function analysis of spontaneous fluctuations in radial artery blood pressure (indwelling catheter) and resulting changes in middle cerebral artery blood flow velocity (transcranial Doppler) in 21 active individuals at sea level upon arrival at 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon re-exposure to 5,260 m after 7 days at 1,525 m (POST7). The Lake Louise Questionnaire was used to evaluate AMS symptom severity. CA was impaired upon arrival at ALT1 ($P < 0.001$) and did not change with acclimatization at ALT16 or upon re-exposure at POST7. CA was not associated with AMS symptoms (all $R < 0.50$, $P > 0.05$). These findings suggest that alterations in CA are an intrinsic consequence of hypoxia and are not directly related to the occurrence or severity of AMS.

transcranial Doppler; cerebral blood flow; cerebral oxygenation; transfer function analysis; hypoxia

CEREBRAL AUTOREGULATION (CA) is a general term used to describe dynamic myogenic, neurologic, and metabolic responses that adjust cerebrovascular resistance to maintain relatively constant cerebral blood flow across a wide range of perfusion pressures (25). Dynamic CA is said to be impaired if fluctuations in mean arterial blood pressure (ABP) lead to concurrent fluctuations in mean cerebral blood flow. Impairments in CA are associated with cerebrovascular disorders (3, 24, 31), yet the relative importance of CA in the development and course of certain pathologies is unclear.

Our initial interest in CA stemmed from the hypothesis that impaired CA may be involved in the development of acute mountain sickness (AMS), high-altitude headache, and cerebral edema (5, 7, 9, 16, 37). Conversely, we showed that impairments in CA upon acute exposure to hypobaric hypoxia preceded, but were not associated with, the development of AMS (2, 33, 35). Furthermore, since several cross-sectional studies demonstrated that impairments in CA persist from 1 to 30 days of high-altitude exposure (1, 2, 11, 12, 17)—when AMS is not present—and are evident in healthy, permanent high-altitude residents (12, 13), it seems reasonable to suggest that a shift in CA may be an inherent and relatively benign consequence of hypoxemia.

To date, no longitudinal studies have characterized CA and tested its relation with AMS during acute and chronic high-altitude exposures. Previous studies have either omitted CA measurements upon arrival at high altitude (7, 11, 17) or followed slow ascent profiles that allow for partial acclimatization before initial measurements (1, 12, 39). In this study, we present novel data from sea-level (SL) residents who rapidly ascended to high altitude (5,260 m; ALT1), acclimatized for 16 days (ALT16), and were subsequently re-exposed to high altitude after spending 7 days at low altitude (1,525 m; POST7). Specifically, we tested the hypotheses that CA would be: *1*) impaired upon rapid ascent to high altitude, *2*) unaffected by 16 days of acclimatization, *3*) unaffected upon re-exposure to the same altitude, and *4*) unrelated to the occurrence or severity of AMS.

METHODS

Study overview. This study was conducted as part of the AltitudeOmics project. Briefly, institutional ethics approval was obtained from the Universities of Colorado and Oregon and the U.S. Department of Defense Human Research Protection Office. Young, healthy SL residents were recruited from the greater Eugene, Oregon, area (elevation 128 m) and screened to exclude anyone who was born or had lived at altitudes >1,500 m for more than 1 yr or had traveled to altitudes >1,000 m in the past 3 mo. After obtaining written consent, physical exams and the Army Physical Fitness Test (push-ups, sit-ups, and a 3.2-km run) were performed to verify health and fitness status. Approximately 4 wk following SL measurements in Eugene, Oregon, subjects were flown to La Paz, Bolivia. They spent two nights at low altitude (1,525 m in Coroico, Bolivia) before being driven to the Chacaltaya Research Station at 5,260 m, while breathing supplemental oxygen. Acute responses to high altitude were assessed ~4 h after arrival and cessation of supplemental oxygen (ALT1). Subjects acclimatized to altitudes ranging from 3,800 to 5,260 m over the next 15 days, with most of the time (75%) spent at 5,250 m. On the 16th day (ALT16), measurements were repeated at 5,260 m before subjects were driven down to Coroico for either 7 or 21 days. Subjects were driven back to the laboratory at 5,260 m for POST7 or POST21 re-exposure measurements.

This report focuses on novel data regarding resting CA, evaluated immediately before a series of cerebrovascular, respiratory, and exercise interventions, as outlined elsewhere (32). We have carefully

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avoided replication of data among reports, except where common variables were necessary to describe subjects' basic physiologic status at the time points of interest [e.g., heart rate (HR), blood pressure, arterial blood gases].

Subjects. We studied 21 subjects at SL (12 men and nine women; 21 ± 1 yr old). Because of logistical problems upon arrival in Bolivia, complete data sets were not obtained on the first seven subjects upon arrival at ALT1. Since the first seven subjects comprised the cohort studied at POST21, longitudinal assessments of CA were limited to the remaining 14 subjects who completed the study at POST7.

Physiology protocol. All subjects were familiarized with study procedures during a practice session at least 48 h before experimental testing at SL. Subjects followed standardized exercise and dietary regimens for 24 h before each measurement period. At each time point, a 22-gauge catheter was inserted into a radial artery at least 1 h before instrumentation. Subjects were seated in an upright position for 15 min, while sensors were placed to measure physiologic variables of interest. Limb lead electrodes were used to measure ECG (Bio Amp; ADInstruments, Colorado Springs, CO). ABP was monitored via a fluid-filled pressure transducer (Deltran II; Utah Medical Products, Midvale, UT) attached to the radial artery catheter. Core temperature was recorded telemetrically from an ingested pill (CorTemp; HQInc, Palmetto, FL). Cerebral blood flow velocity (CBFv) in the left middle cerebral artery (MCA) was measured by transcranial Doppler (2 MHz; Spencer Technologies, Seattle, WA) at depths ranging from 43 to 54 mm. Signal quality was optimized, and an M-mode screen shot was recorded to facilitate subsequent probe placements and insonation angles.

After verification of signal quality, resting data were recorded for 6 min, while subjects breathed room air to assess CA at each altitude. Continuous analog data [ABP, CBFv, ECG, oxygen (O_2), and carbon dioxide (CO_2)] were recorded at 200 Hz (PowerLab 16/30; ADInstruments) for offline analysis. Core temperature and arterial blood samples (2 ml) were taken during the last 30 s of measurement periods. Blood samples were taken from the radial artery catheter, and blood gases were analyzed for partial pressure of arterial CO_2 (Pa_{CO_2}) and partial pressure of arterial O_2 (Pa_{O_2}) in triplicate (RAPIDLab 248; Siemens, Erlangen, Germany) and corrected for body temperature (15, 29).

Acute mountain sickness. Self-reported sections of the Lake Louise Questionnaire (LLQ) were used to assess AMS on ALT1 and POST7 (~12 h after arrival). Moderate and severe AMS was defined as LLQ ≥ 3, and LLQ ≥ 6, including headache, respectively (27).

Data analysis. Transfer function analyses were used to assess dynamic CA, based on spontaneous fluctuations in the raw ABP and CBFv signals, as described previously (33, 34). Briefly, 6-min recordings of instantaneous ABP and CBFv were reduced to beat-by-beat averages, resampled at 5 Hz, and transformed from the time-to-frequency domain using fast Fourier transformations (512 points/segment with 40% overlap). The transfer function from mean ABP to CBFv was expressed in terms of coherence, gain, and phase shift in the very low frequency range (0.02–0.07 Hz), where dynamic CA is most active (21, 22), as well as in low (0.07–0.20 Hz) and high (0.20–0.35 Hz) frequency ranges. All data were used in subsequent statistical analyses. Reduction in phase shift was considered the primary criterion for impaired CA, because it signifies shorter delay in transmission of pressure (ABP) into flow (CBFv) or a reduction in the ability of the cerebrovascular system to buffer changes in ABP and maintain consistent blood flow. Yet, since increases in gain (increase in CBFv relative to a change in ABP) and coherence (linear correlation between ABP and CBFv) may also suggest CA impairment (8, 24, 41), all three transfer function metrics are reported. To address difficulties in interpreting possible permutations of these three variables, the inverse transfer function of the resulting gain and phase shift was used to express results in the time domain as a step function that could be fitted to one of 10 curves representing a single autoregulation index (ARI) score (36). An ARI score of zero indicates complete lack of autoregulation, and nine indicates perfect autoregulation.

Statistics. After calculating descriptive statistics (mean ± SD) and verifying normality (D'Agostino and Pearson tests), variables were analyzed by repeated-measures ANOVA to evaluate the effect of time on CA metrics with Fisher's least significant difference post hoc tests and the Holm procedure to correct for multiple comparisons (α = 0.05).

Spearman ρ correlations were run to evaluate relations between CA metrics and the severity of LLQ symptom scores. Specifically, we tested the ability of CA assessments, measured at SL and upon arrival at ALT1, to predict ensuing symptoms of AMS (7). Also, because AMS classification is dichotomous (i.e., positive vs. negative), we used receiver-operating characteristic (ROC) analyses (14, 18) to evaluate the sensitivity (true positive rate) and specificity (true negative rate) of the ability of ARI scores to detect mild and severe AMS. The ROC area under the curve (AUC) statistic was used as an indicator of test accuracy. An AUC of 1.0 signifies a perfect test, with no chance of false-positive or false-negative results, whereas an AUC of 0.5 signifies a meaningless test, where the probability of identifying a true positive result is only 50%.

RESULTS

Effect of rapid ascent to high altitude. At SL, resting cardiovascular (HR, ABP, CBFv) and CA (coherence, gain, phase shift, and ARI scores) measurements were characteristic of young, healthy individuals with intact CA (Table 1 and Fig. 1). From SL to ALT1, Pa_{O_2} and Pa_{CO_2} decreased (65% and 26%, respectively; $P < 0.001$; Table 1). This degree of hypoxia increased HR ($P < 0.001$) but did not affect mean ABP or CBFv. Very low frequency power spectral density of ABP and

Table 1. *Resting data*

Variable		SL	ALT1	ALT16	POST7
Pa_{O_2}	mmHg	103 ± 5	36 ± 3*	45 ± 4*†	42 ± 4*†‡
Pa_{CO_2}	mmHg	37 ± 4	28 ± 2*	21 ± 3*†	24 ± 3*††
HR	beats/min	73 ± 9	90 ± 18*	95 ± 12*	85 ± 15*‡
ABP	mmHg	77 ± 6	76 ± 14	81 ± 10	76 ± 8
CBFv	cm/s	62 ± 9	63 ± 14	59 ± 7	57 ± 9
PSD ABP	$mmHg^2/Hz$	11 ± 13	9 ± 4	9 ± 5	6 ± 4
PSD CBFv	$(cm/s)^2/Hz$	13 ± 19	14 ± 16	10 ± 6	11 ± 8
Coherence		0.42 ± 0.12	0.64 ± 0.15*	0.70 ± 0.16*	0.55 ± 0.12*‡
Gain	%/%	0.64 ± 0.24	0.88 ± 0.35*	0.85 ± 0.25*	0.97 ± 0.33*
Phase shift	radians	0.48 ± 0.28	0.17 ± 0.21*	0.27 ± 0.09*	0.25 ± 0.19*
ARI		4.4 ± 1.0	2.8 ± 0.9*	2.8 ± 1.0*	3.3 ± 1.6*

*Different from sea level (SL); †different from arrival at 5,260 m (ALT1); ‡different from after 16 days of acclimatization (ALT16). n = 14; mean ± SD. POST7, re-exposure to 5,260 m after 7 days at 1,525 m; Pa_{O_2}, partial pressure of arterial oxygen; Pa_{CO_2}, partial pressure of arterial carbon dioxide; HR, heart rate; ABP, arterial blood pressure; CBFv, cerebral blood flow velocity; PSD, power spectral density; ARI, autoregulation index.

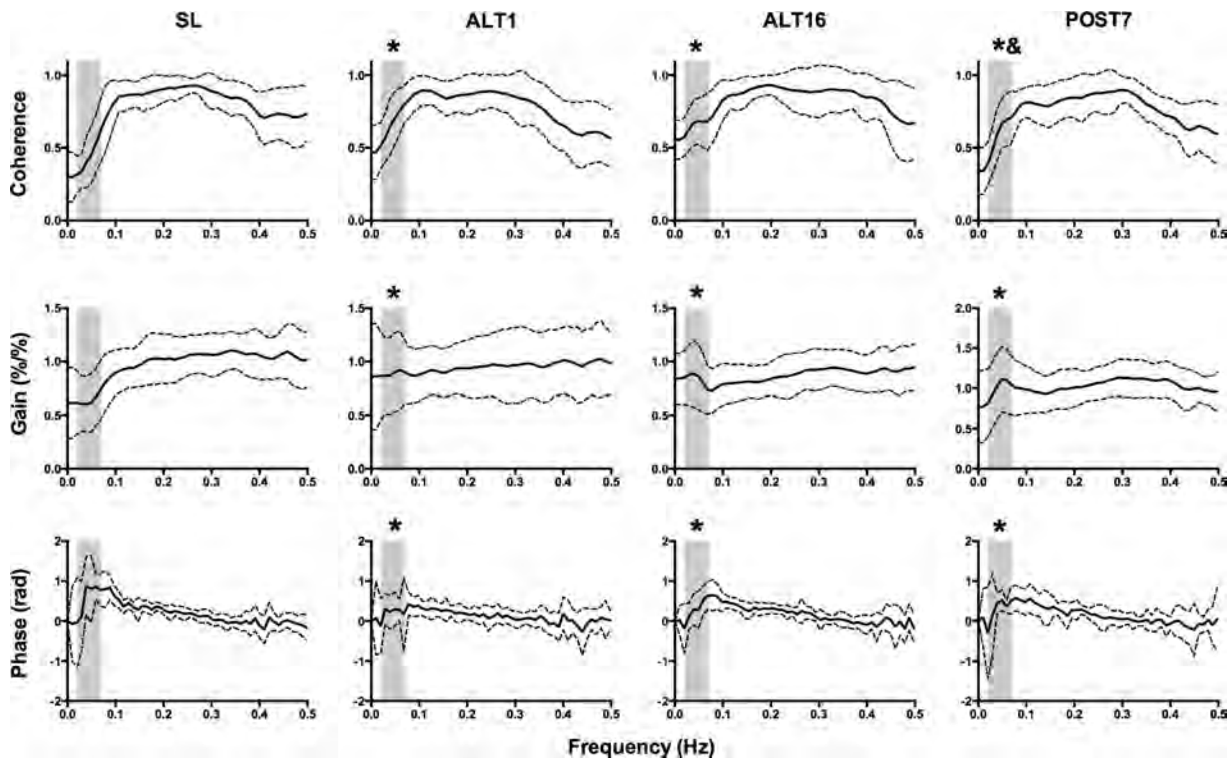

Fig. 1. Arterial blood pressure to cerebral blood flow velocity transfer function metrics (mean ± SD from 0 to 0.5 Hz) at sea level (SL), upon arrival at 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon re-exposure to 5,260 m after 7 days at low altitude (POST7). Similar impairments in cerebral autoregulation (increased coherence and gain and decreased phase shift) from SL were seen in the very low frequency (0.02–0.07 Hz; shaded areas) at ALT1, ALT16, and POST7 ($P < 0.05$). rad, radians. *Different from SL; &different from ALT16.

CBFv was unaltered, but increases in transfer function coherence ($P < 0.001$) and decreases in phase shift ($P < 0.050$) and ARI score ($P < 0.001$) were consistent (in 13 of 14 subjects) with the definition of impaired CA at ALT1.

Effect of acclimatization to high altitude. Acclimatization increased resting Pa_{O_2} (27%) and decreased Pa_{CO_2} (22%) from ALT1 to ALT16 (both $P < 0.001$), without affecting HR, ABP, or CBFv. Measures of CA at ALT16 were unchanged from ALT1 and remained impaired relative to SL in the very low frequency range (all $P < 0.010$; Table 1 and Fig. 1).

Effect of re-exposure to high altitude. Resting Pa_{O_2} and Pa_{CO_2} at POST7 fell between ALT1 and ALT16 values (all $P < 0.050$ vs. ALT1 and vs. ALT16), indicating that the degree of acclimatization achieved at ALT16 was partially maintained at POST7. Assessments of CA at POST7 were similar to those at ALT1 and ALT16 and remained impaired relative to SL in the very low frequency range ($P < 0.050$; Table 1 and Fig. 1).

Association between CA and AMS. Of the 21 subjects, 17 reported symptoms of at least moderate AMS at ALT1 (LLQ = 6.4 ± 2.2), 10 of who met the criteria for severe AMS (LLQ = 7.8 ± 1.7). Correlations among CA metrics preceding the development of AMS symptom were weak (all $r < 0.50$, $P > 0.050$; Fig. 2). The ROC analysis revealed that ARI scores measured at SL were not sensitive or specific predictors of moderate (AUC = 0.54, $P = 0.788$) or severe (AUC = 0.69, $P = 0.139$) AMS. Additionally, the degree of impairment in CA (measured as the change in ARI from SL to ALT1) was not a sensitive or specific predictor of moderate (AUC = 0.53, $P = 0.881$) or severe (AUC = 0.72, $P = 0.124$) AMS. None of the 14 subjects studied at POST7 reported symptoms of AMS; thus associations with CA could not be tested.

DISCUSSION

The key findings of this study were that CA, as assessed by transfer function analysis, is *1*) impaired upon rapid ascent to high altitude, *2*) unaffected by acclimatization or *3*) subsequent re-exposure to the same altitude, and *4*) not a sensitive or specific predictor of AMS. Based on our results, we question whether the so-called impairment in CA that persists at high altitude is characteristic of pathological insufficiency in cerebrovascular regulation (16) or alternatively, reflects a relatively benign relaxation in autoregulation.

Effect of high altitude on CA. This is the first longitudinal study of CA at high altitude—from rapid ascent through acclimatization and upon re-exposure after a short period at low altitude. We show that impairment of CA was a consistent characteristic across this high-altitude exposure profile.

Increased transfer function coherence and gain, along with reduced phase shift and ARI score upon rapid ascent, were all consistent with the classic definition of impaired CA (Table 1) and outside the normal range of expected variability (6), implying that changes in ABP were transmitted more readily into the cerebral circulation as changes in CBFv at high altitude. Our finding of impaired CA after <1 day of travel from low to high elevation is consistent with our previous findings after 4 h in a hypobaric chamber (35) and fills an important gap in the literature between studies conducted in laboratories with hypoxic gas mixtures, where normobaric

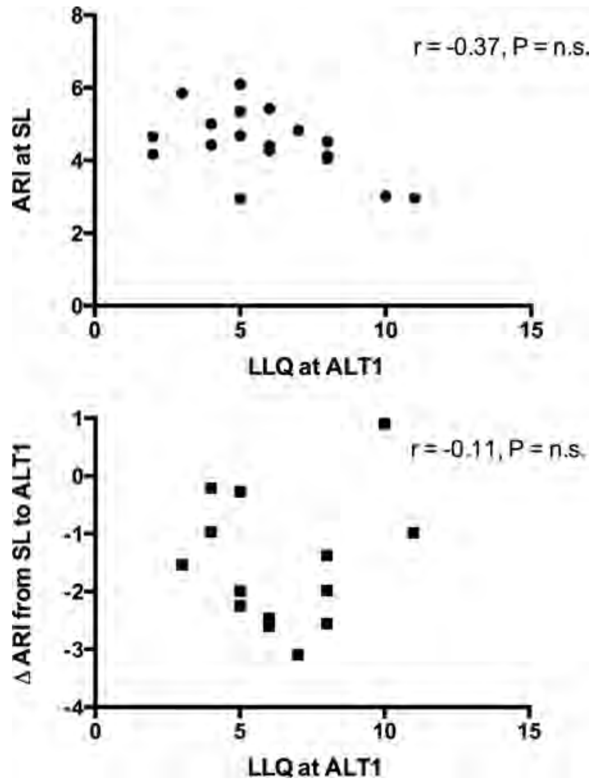

Fig. 2. Scatter plots showing no relation ($P > 0.05$) between autoregulation indices (ARI), measured at SL (*top*) and as the change (Δ) from SL to arrival at high altitude (ALT1; *bottom*), and acute mountain sickness symptoms' scores from the Lake Louise Questionnaire (LLQ) at ALT1.

hypoxia was achieved in a matter of minutes (5, 10, 26, 34), and studies of trekkers, where several days of progressive ascent preceded initial high-altitude measurements (1, 2, 12, 37). Impaired CA at rest in acute hypoxia is a consistent finding among all but one study (26), suggesting that neither the mode nor rate of ascent appears to affect the general assessment.

By evaluating CA upon initial exposure and after 16 days at high altitude, we were able to determine if changes in CA occur with acclimatization, as might be expected with increased Pa_{O_2} (2, 35), decreased Pa_{CO_2} (19, 23, 26), and further sympathoexcitation (1). On the contrary, we found no change in CA over the course of acclimatization (Table 1). Our longitudinal findings are consistent with other cross-sectional studies, demonstrating impaired CA at various time points after arrival at high altitude (1, 2, 7, 11, 12, 37) and in permanent high-altitude residents (12, 13). These results may indicate that assessments of CA are less sensitive to changes in Pa_{O_2} and Pa_{CO_2} near their respective extremes. Alternatively, a slight improvement in CA, due to increased Pa_{O_2} (2, 35), may have been masked if the opposing effects of Pa_{CO_2} (19, 23, 26) and/or sympathoexcitation (1) on CA were heightened over time at altitude. Further testing with manipulation of arterial gases and sympathetic activity is necessary to determine the relative influence of arterial gases and neural stimulation on CA at high altitude, yet impaired CA remains a consistent, functional consequence across time at high altitude.

As an additional test of the hypothesis that impaired CA is a consistent response to hypoxemia, we sent subjects down to low altitude for 7 days and re-evaluated their CA response after a second rapid ascent back to high altitude. Upon re-exposure, the measured impairment in CA was similar to that observed upon the first ascent (ALT1) and after acclimatization (ALT16). Together, these results demonstrate that impaired autoregulation was a consistent characteristic of hypoxemia across our study and imply that slow fluctuations in arterial pressure were dampened less effectively by the cerebral vasculature, regardless of the state of acclimatization. What remains to be determined is if such a tenuous pressure-flow relation may be potentially harmful.

Relation of CA to AMS. Impairment of CA has been suggested to play a role in the development of AMS by either permitting cerebral overperfusion and mechanical disruption of the blood brain barrier (i.e., vasogenic cerebral edema) when mean ABP is elevated or by cerebral underperfusion and exacerbation of cerebral hypoxia/ischemia when mean ABP is lowered (9, 16). In the present study, we found no correlation between measures of CA and subsequent AMS symptom scores (Fig. 2), which opposes the notion that lower CA predisposes people to AMS or conversely, that higher CA confers protection from AMS. Our additional ROC analyses of AMS status confirmed that ARI scores were neither sensitive nor specific indicators for the development of moderate or severe AMS upon arrival at high altitude. These findings are congruent with our previous report following the time course of changes in CA and AMS symptoms over the first 10 h of exposure to hypobaric hypoxia (35), where we found similar levels of CA impairments in subjects who eventually developed AMS or stayed healthy, but are at odds with other studies showing some association between CA and AMS symptoms (5, 37). Our data also counter a recent finding that SL assessments of CA predict ensuing severity of AMS (7).

Discrepancies among studies may be explained by the various methods used to assess CA (transfer function vs. leg cuff; see Limitations below), the questionnaires used to assess AMS (LLQ vs. Environmental Symptoms Questionnaire), and the statistical approach used to evaluate the relation between CA and AMS (correlation vs. ROC). We acknowledge that caution should be exercised when interpreting correlations with an ordinal-level variable, such as the LLQ score, because by definition, the scale has limited mathematical meaning. For example, a LLQ score of six does not imply that symptom severity is exactly twice that of a score of three. Due to the intrinsic level of measurement, we believe that LLQ scores are best restricted to dichotomous classification of positive or negative AMS status and thus place more emphasis on the negative results of our ROC analysis. We encourage others to consider this method of analysis for future AMS studies.

Overall, given the similarity in CA responses among individuals with a wide range of AMS scores, we do not believe that changes in CA cause AMS. This assertion is supported further by the complete lack of association between impaired CA at POST7 when no symptoms of AMS were reported and previous reports documenting impaired CA in healthy, high-altitude natives (12, 13). Nonetheless, we must acknowledge that the alteration in CA upon acute altitude exposure may set up a tenuous pressure-flow relation that could permit AMS to develop if other, yet-unidentified factors are present at the same time.

Since impairment of CA appears to be a consistent physiological response in hypoxic environments and unrelated to AMS status, it is tempting to speculate that the underlying change in the cerebral pressure-flow relation may actually promote successful acclimatization or adaptation to chronic states of hypoxemia (4). It is possible that impairment of CA could promote cerebral oxygen delivery in a time of need, since it allows greater cerebral perfusion for a given increase in ABP. This potentially beneficial consequence of impaired CA during hypoxemic stress might outweigh the relative risk of reduced cerebral perfusion if ABP were to drop. We therefore raise the possibility that the term "impaired CA" may be a misnomer, because it implies an association with pathology that has yet to be substantiated in acute or chronic hypoxemia. We suggest that "relaxation of CA" might be a more accurate term to describe changes in the cerebral pressure-flow relation from normoxia to hypoxia in the absence of pathology.

Limitations. One major limitation affecting the field is the lack of a gold-standard method to assess CA. We have chosen to evaluate rhythmical fluctuations in CA via transfer function analysis, primarily because we believe it captures the natural cerebral pressure-flow relation over time and thus has greater practical relevance over methods that induce larger, more abrupt changes in ABP, as with leg-cuff inflation/deflation, rapid tilting, or more sustained changes in ABP, such as with pharmaceutical interventions. Still, we acknowledge that transfer function analysis of resting data monitors relatively subtle fluctuations in ABP and CBFv, which if amplified, may not show impairment in CA (39). These factors may limit the generalizability of resting CA assessments and lead to an overstatement of the clinical relevance of the findings. Additionally, there are no universal standards for the parameter settings used in transfer function analysis or interpretation of subsequent results, which makes comparisons among studies problematic. Future work is needed to clarify differences in methods used to assess CA in hypoxemic states and evaluate if these changes are generalizable to clinical settings.

Most CA studies rely on transcranial Doppler measurements of flow velocity and assume that vessel diameter is unchanged; yet, there is evidence to suggest that this assumption may be invalid at extreme altitudes (39, 40). Dilation of the MCA at ALT1 may explain why MCA velocity did not follow the expected increase in CBF upon acute exposure to high altitude (30). We do not believe potential MCA dilation affected our interpretation, because the phase shift—our primary criterion for assessing changes in CA—measures the relative timing of oscillations in ABP and CBFv and thus is largely independent of absolute flow. However, since small changes in diameter can have profound effects on flow (flow $\sim$ radius^4), future studies must consider the use of continuous flow measurements, instead of velocity measurements, to assess CA accurately in hypoxia.

Finally, our measurements of CA were limited to the MCA and relied on pressure measurements taken in the radial artery. Since regional differences in cerebrovascular regulation have been reported recently (20, 28, 38), more specific measurements of regional pressure and flow are needed to characterize CA fully.

Conclusions. Our data demonstrate that the initial impairment of CA upon acute exposure to high altitude is invariant with acclimatization and re-exposure, suggesting that relaxation in the regulation of the cerebral pressure-flow relation is a characteristic response to hypoxia that is unaffected by the degree of acclimatization. Since changes in CA do not follow the progression and resolution of AMS, we question the clinical relevance of impaired CA at high altitude.

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DISCLOSURES

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Author contributions: A.W.S., J-L.F., O.E., B.K., C.G.J., A.T.L., R.B.P., and R.C.R. conception and design of research; A.W.S., J-L.F., O.E., and N.B. performed experiments; A.W.S., O.E., and N.B. analyzed data; A.W.S., J-L.F., B.K., R.B.P., and R.C.R. interpreted results of experiments; A.W.S. prepared figures; A.W.S. drafted manuscript; A.W.S., J-L.F., O.E., N.B., B.K., C.G.J., A.T.L., R.B.P., and R.C.R. edited and revised manuscript; A.W.S., J-L.F., O.E., N.B., B.K., C.G.J., A.T.L., R.B.P., and R.C.R. approved final version of manuscript.

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HIGHLIGHTED TOPIC | *Hypoxia*

AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high-altitude acclimatization and reexposure

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[1]*Institute of Sports Sciences, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland;* [2]*Lemanic Neuroscience Doctoral School, University of Lausanne, Lausanne, Switzerland;* [3]*Altitude Research Center, Department of Emergency Medicine, University of Colorado Denver, Aurora, Colorado;* [4]*Department of Biology; University of Colorado, Colorado Springs, Colorado; and* [5]*Department of Human Physiology, University of Oregon, Eugene, Oregon*

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Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT, Roach RC. AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high-altitude acclimatization and reexposure. *J Appl Physiol* 116: 911–918, 2014. First published December 19, 2013; doi:10.1152/japplphysiol.00704.2013.—The present study is the first to examine the effect of high-altitude acclimatization and reexposure on the responses of cerebral blood flow and ventilation to CO_2. We also compared the steady-state estimates of these parameters during acclimatization with the modified rebreathing method. We assessed changes in steady-state responses of middle cerebral artery velocity (MCAv), cerebrovascular conductance index (CVCi), and ventilation ($\dot{V}_E$) to varied levels of CO_2 in 21 lowlanders (9 women; 21 ± 1 years of age) at sea level (SL), during initial exposure to 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon reexposure to altitude following either 7 (POST7) or 21 days (POST21) at low altitude (1,525 m). In the nonacclimatized state (ALT1), MCAv and $\dot{V}_E$ responses to CO_2 were elevated compared with those at SL (by 79 ± 75% and 14.8 ± 12.3 l/min, respectively; $P = 0.004$ and $P = 0.011$). Acclimatization at ALT16 further elevated both MCAv and $\dot{V}_E$ responses to CO_2 compared with ALT1 (by 89 ± 70% and 48.3 ± 32.0 l/min, respectively; $P < 0.001$). The acclimatization gained for $\dot{V}_E$ responses to CO_2 at ALT16 was retained by 38% upon reexposure to altitude at POST7 ($P = 0.004$ vs. ALT1), whereas no retention was observed for the MCAv responses ($P > 0.05$). We found good agreement between steady-state and modified rebreathing estimates of MCAv and $\dot{V}_E$ responses to CO_2 across all three time points ($P < 0.001$, pooled data). Regardless of the method of assessment, altitude acclimatization elevates both the cerebrovascular and ventilatory responsiveness to CO_2. Our data further demonstrate that this enhanced ventilatory CO_2 response is partly retained after 7 days at low altitude.

cerebral blood flow; cerebral CO_2 reactivity; rebreathing; altitude acclimatization

THE ABILITY TO MAINTAIN ADEQUATE oxygen transport to the brain by cerebral blood flow (CBF) in hypoxic environments is vital. The CBF responsiveness to CO_2, termed cerebrovascular CO_2 reactivity, provides a useful, noninvasive index of cerebrovascular function (3, 19). To date, only a handful of studies have investigated the effect of acclimatization to high altitude on cerebrovascular CO_2 reactivity (1, 16, 17, 24, 30, 49). It is difficult to interpret the findings from these studies due to the timing of measurements at high altitude (1, 16, 17, 24, 25), the confounding effects of previous high altitude exposure (1), artificial normobaric hypoxia (28, 46), and the method used to assess reactivity (24, 30, 49). Data obtained by Fan et al. (16, 17) on subjects at different stages of altitude acclimatization suggest that cerebrovascular CO_2 reactivity is elevated with prolonged exposure to high altitude when using a modified rebreathing technique. In contrast, Lucas et al. (30) reported, using a steady-state technique (poikilocapnic hypoxia), reduced cerebrovascular CO_2 reactivity in the same subjects assessed at the end of a 14-day stay at 5,050 m. More recently, Rupp et al. (49) reported a reduced cerebrovascular CO_2 reactivity during steady-state hypoxic hypercapnia following 5 days at 4,350 m. Thus the effect of altitude acclimatization on cerebrovascular CO_2 reactivity remains unclear.

In addition, it is unknown whether and for how long changes in cerebrovascular CO_2 reactivity from acclimatization persist after descent. Repetitive 7-mo exposures to high altitude were reported to improve arterial O_2 saturation (Sa_{O_2}), lower resting heart rate (HR), and decrease susceptibility to acute mountain sickness (AMS) upon subsequent reexposures (59). Remarkably, these prior exposure adaptations persisted despite a 5-mo deacclimatization period. The specific effect of high-altitude reexposure on cerebrovascular and ventilatory responsiveness to CO_2 has yet to be examined.

Changes in cerebrovascular CO_2 reactivity with high-altitude acclimatization depend on the method of assessment. At sea level, the steady-state method results in higher cerebrovascular CO_2 reactivity (40–42) and lower ventilatory CO_2 sensitivity (6, 18, 23, 55) compared with the modified rebreathing test. These differences have been attributed to the presence of a P_{CO_2} gradient (between alveolar, arterial, and cerebrospinal fluid compartments) during the steady-state method, which is supposedly abolished or minimized during rebreathing (6). Meanwhile, elevated basal $\dot{V}_E$ and subsequent underestimation of the ventilatory CO_2 sensitivity has been proposed as one possible explanation for lower steady-state estimates (34). No studies have directly compared the steady-state and modified rebreathing test estimates of cerebrovascular and ventilatory CO_2 responsiveness following ascent or acclimatization to high altitude.

The purpose of the present study was therefore twofold: first, we wished to assess the effect of altitude exposure on cerebro-

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vascular and ventilatory responsiveness to CO_2 in acute conditions after acclimatization and upon reexposure to high altitude after a period spent at low altitude; second, we wished to compare the steady-state and modified rebreathing methods for assessing the ventilatory and cerebrovascular responsiveness to CO_2 at high altitude.

METHODS

Subject Recruitment and Screening

This study was conducted as part of the AltitudeOmics project. Following institutional ethics approval, young (19–23 years old), healthy, sea-level residents were recruited from the greater Eugene, Oregon, area (elevation 130 m). Potential subjects were screened to exclude anyone who was born or had lived at altitudes >1,500 m for more than 1 year or had traveled to altitudes >1,000 m in the past 3 mo. A detailed description of subject recruitment procedures, including inclusion and exclusion criteria, has been presented elsewhere (54).

Ethical Approval

The study was performed according to the Declaration of Helsinki and was approved by the institutional review boards of the University of Colorado and the University of Oregon, and by the Human Research Protection Office of the U.S. Department of Defense. All participants were informed regarding the procedures of this study, and written informed consents were obtained prior to participation.

Experimental Design

After familiarization with the experimental procedures outlined below (*visit 1*), the subjects underwent experimental trials near sea level (SL) (130 m; barometric pressure 749 mmHg) and three times at high altitude (5,260 m, Mt. Chacaltaya, Bolivia; barometric pressure 406 mmHg) on the 1st and 16th days at high altitude (ALT1 and ALT16, respectively), and again after either 7 (POST7; $n = 14$) or 21 (POST21; $n = 7$) days at low altitude (1,525 m; barometric pressure 639 mmHg). An overview of the entire experimental design and protocol has been described in detail elsewhere (54).

Experimental Protocol

For each subject, all ALT measurements were carried out around the same time of day to minimize any confounding effect of circadian rhythm. Measurements were taken upon arrival at ALT1 to minimize the influence of AMS. Likewise, no symptoms of AMS were observed at ALT16 or POST7.

For this study, following 10–15 min of quiet rest in a seated position, each experimental testing session consisted of *1*) instrumentation, *2*) 10 min in room air at baseline, and *3*) cerebrovascular CO_2 reactivity tests. The cerebrovascular CO_2 reactivity tests consisted of *1*) 10 min with end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) clamped at 40 mmHg; *2*) 3 min of voluntary hyperventilation to lower $P_{ET_{CO_2}}$ to ~20 mmHg; *3*) the modified rebreathing test (details below); and *4*) 3 min with $P_{ET_{CO_2}}$ clamped at 50 mmHg. The entire cerebrovascular CO_2 reactivity protocol was carried out in a background of hyperoxia (end-tidal P_{O_2} [$P_{ET_{O_2}}$] >250 mmHg).

Experimental Setup

Throughout the protocol, the subjects sat upright and breathed through a mouthpiece attached to a two-way, nonrebreathing valve (Hans-Rudolph 2700, Hans-Rudolph, Shawnee, KS). The breathing circuit allowed switching from room air to either an end-tidal clamping system or a rebreathing system. The end-tidal clamping setup used in the present study is a modified version of the system previously described by Olin et al. (39). The setup allowed stabilizing $P_{ET_{CO_2}}$ at 40 and 50 mmHg. Throughout the end-tidal P_{CO_2} clamping, we maintained $P_{ET_{O_2}}$ at >250 mmHg by titrating 50% or 100% O_2 into the inspiratory reservoir at SL and ALT, respectively.

Modified Rebreathing Method

The modified rebreathing method is well established for assessing both ventilatory and cerebrovascular CO_2 reactivities (14, 16, 34, 41). By using hyperoxia ($P_{ET_{O_2}}$ >250 mmHg) the test minimizes the output of peripheral chemoreceptors (11, 21), and the ventilatory response to the modified rebreathing method can thus be interpreted as the ventilatory CO_2 sensitivity primarily from the central chemoreflex. The details of the modified rebreathing method have been previously described in Fan al. (16, 17). The rebreathing bag was filled with gas to achieve inspired P_{CO_2} and P_{O_2} of 0 mmHg and 300 mmHg, respectively, at each altitude. Subjects were instructed to hyperventilate for 3 min (*part 2*) to lower and then maintain $P_{ET_{CO_2}}$ at 20 mmHg at both sea level and 5,260 m (in background $P_{ET_{O_2}}$ >250 mmHg). Subjects were then switched to the rebreathing bag, and following two initial deep breaths to mix the gas from the bag with that in the respiratory system, they were instructed to breathe ad libitum (*part 3*). The rebreathing tests were terminated when $P_{ET_{CO_2}}$ reached 50 mmHg, $P_{ET_{O_2}}$ dropped below 200 mmHg, or the subject reached the end of his or her hypercapnic tolerance.

Measurements

Cerebrovascular variables. Middle cerebral artery velocity (MCAv, an index of cerebral blood flow) was measured in the left middle cerebral artery using a 2-MHz pulsed Doppler ultrasound system (ST3; Spencer Technology, Seattle, WA). The Doppler ultrasound probe was positioned over the left temporal window and held in place with an adjustable plastic headband (Marc 600 Headframe; Spencer Technology). The signal was acquired at depths ranging from 43 to 54 mm. Signal quality was optimized, and an M-mode screen shot was recorded to facilitate subsequent probe placements. Peripheral saturation was measured on the right side of the forehead by pulse oximetry (N-200; Nellcor, Hayward, CA).

Cardiovascular variables. Beat-to-beat mean arterial blood pressure (MAP) was measured from an arterial catheter inserted in a radial artery, and connected to a calibrated, fluid-filled, disposable pressure transducer positioned at the level of the heart (DELTRAN II; Utah Medical, Salt Lake City, UT). HR was determined using three-lead electrocardiography systems (ADInstruments BioAmp & Micromaxx; SonoSite, Bothell, WA). Cerebrovascular conductance index (CVCi) was calculated using the equation CVCi = MCAv/MAP and normalized to values obtained at a $P_{ET_{CO_2}}$ of 20 mmHg, and expressed as percentage change.

Respiratory variables. $\dot{V}_E$ was measured using a pneumotachograph (Universal Ventilation Meter; Vacu·Med, Ventura, CA; Ultima series; Medgraphics CPX, Minneapolis, MN) and expressed in units adjusted to body temperature and pressure, saturated (BTPS). $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ were measured using fast-responding gas analyzers (O_2Cap Oxygen analyzer; Oxigraf, Mountain View, CA). The pneumotachograph was calibrated using a 3-liter syringe (Hans-Rudolph 5530) and the gas analyzers were calibrated using gas mixtures of known concentrations of O_2 and CO_2 prior to each testing session.

Arterial blood gas variables. An arterial catheter (20–22 gauge) was placed into a radial artery and blood samples (2 ml) were taken over approximately five cardiac cycle periods. Core body temperature was telemetrically recorded from an ingested pill (CorTemp; HQInc, Palmetto, FL). All samples were analyzed immediately for arterial pH, P_{O_2} (Pa_{O_2}), P_{CO_2} (Pa_{CO_2}) (Rapidlab 248; Siemens Healthcare Diagnostics, Munich, Germany), hemoglobin concentration, and O_2 saturation (Sa_{O_2}) (Radiometer OSM3; Radiometer Medical ApS, Copenhagen, Denmark). The blood gas values were analyzed in triplicate and temperature-corrected (26, 53). Arterial bicarbonate concentration

($[HCO_3^-]$) was subsequently calculated using the Henderson-Hasselbalch equation.

Data Acquisition

All analog data were sampled and recorded at 200 Hz on a personal computer for off-line analysis (Powerlab 16/30; ADInstruments, Bella Vista, Australia).

Data Analysis

Steady-state responses. Because the subjects could not tolerate $P_{ET_{CO_2}}$ clamping at 50 mmHg at ALT16, the steady-state MCAv-CO_2, MAP-CO_2, and CVCi-CO_2 slopes were estimated from the difference in mean MCAv, MAP, and CVCi at the end of 20 and 40 mmHg $P_{ET_{CO_2}}$ clamping (20-s averages) and plotted against the change in Pa_{CO_2} between these two conditions across all time points (SL, ALT1, ALT16, POST7, and POST21). The absolute value of $\dot{V}_E$ at clamp 40 mmHg was used as an estimate of steady-state $\dot{V}_E$ responsiveness to CO_2, because voluntary hyperventilation was necessary to reduce $P_{ET_{CO_2}}$ to 20 mmHg.

Modified rebreathing. The rebreathing data were first reduced to 1-s averages across the entire rebreathing period. The $\dot{V}_E$-CO_2 slopes were analyzed using a specially designed program (Analyse $\dot{V}_E$ Rebreathing programme rev11; University of Toronto, Toronto, ON, Canada) as previously described (15, 16, 34). The MCAv-CO_2 slopes were analyzed using a commercially available graphing program (Prism 5.0d; GraphPad Software, San Diego, CA), whereby segmental linear regression (least squares fit) was used to estimate the MCAv-CO_2 slope during the modified rebreathing. For comparison, we plotted the MCAv-CO_2 slopes using a sigmoid curve as described by Battisti-Charbonney et al. (4) using the Prism program. To minimize the sum of squares for nonlinear regression (Levenberg-Marquardt algorithm) we used the equation $MCAv = a + (b/\{1 + \exp[-(P_{ET_{CO_2}} - c)/d]\})$, where MCAv is the dependent variable in cm/s, $P_{ET_{CO_2}}$ is the independent variable in mmHg, a is the minimum MCAv determined from the mean MCAv of the hypocapnic (hyperventilation) region, b is the maximum MCAv value, c is the midpoint value of MCAv, and d is the range of the linear portion of the sigmoid (inverse reflection of the slope of the linear portion).

We found good agreement in the MCAv-CO_2 slope obtained from these two models ($R^2 = 0.71$). However, due to the range of $P_{ET_{CO_2}}$ used in this study, segmental linear regression generally provided better fit across all conditions, whereas the sigmoidal curve model was the preferred model for only 12 out of 58 trials. As such, only the MCAv-CO_2 slopes obtained using the segmental linear model are presented.

Statistical Analysis

Due to logistical impacts on planning and transportation, not all subjects were able to participate in all high-altitude studies. See the Figs. 1–3 and Table 1 for complete sample size reporting for each procedure. Most data are reported as the improvement over the time of acclimatization (change from ALT1 to ALT16) and as the amount of that improvement that was retained after time at low altitude, calculated as % retention = (POST7 or POST21 − ALT1)/(ALT16 − ALT1)·100 (5). The effects of altitude acclimatization and reexposure (between SL, ALT1, ALT16, POST7, and POST21) on the steady-state MCAv-CO_2 slope, CVCi-CO_2 slope, and $\dot{V}_E$ at 40 mmHg were analyzed using a mixed-model linear regression (IBM SPSS Statistics version 21; IBM, Armonk, NY). To assess the effects of altitude acclimatization (between SL, ALT1, and ALT16) on the rebreathing estimates of MCAv-CO_2 and $\dot{V}_E$-CO_2 slopes, we used mixed-model linear regression analysis (diagonal repeated covariance assumed). The interactions between variables of interest were assessed using correlational (Pearson) analysis (IBM SPSS Statistics version 21). Data are shown as mean ± SD. Results were considered significant at $\alpha < 0.05$. Trends were consider at the $\alpha < 0.10$ level. A priori power calculations ($\alpha = 0.05$, $\beta = 0.20$) were used to determine sample size and limit type II error.

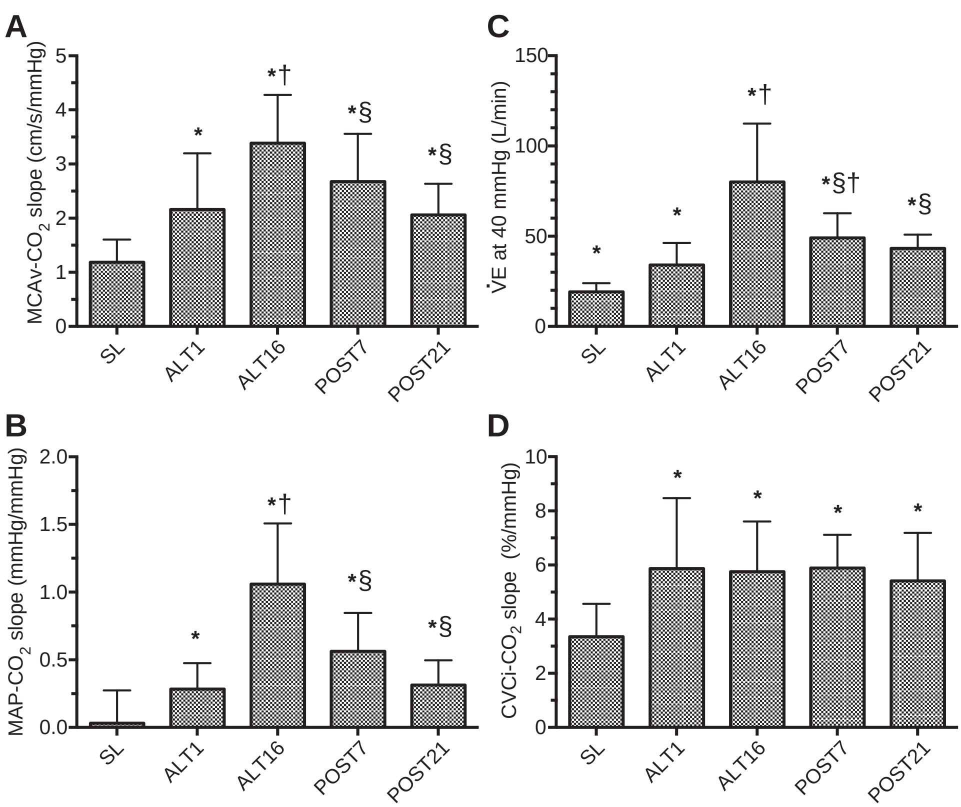

Fig. 1. Changes in steady-state estimates of cerebrovascular, cardiovascular, and ventilatory responsiveness to CO_2 with acclimatization and reexposure to 5,260 m. Values are mean ± SD. *Different from SL ($P < 0.05$); †different from ALT1 ($P < 0.05$), §different from ALT16 ($P < 0.05$).

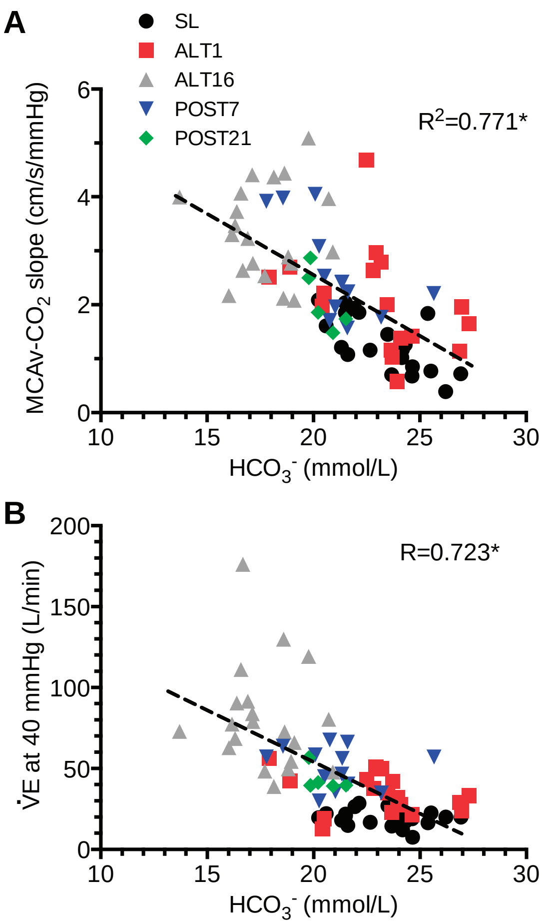

Fig. 2. Relationship between standard basic excess and steady-state cerebrovascular, ventilatory, and cardiovascular responsiveness to CO_2 with acclimatization to altitude. *Significant correlations ($P < 0.05$).

RESULTS

Detailed baseline characteristics of the 21 (9 women; age 21 ± 1 years) subjects participating in AltitudeOmics are presented elsewhere (54). All 21 subjects completed the protocol at SL. Due to logistical issues, 4 of 21 subjects were unable to complete the entire experimental protocol at ALT1. Upon reexposure to altitude, 14 of 14 subjects completed the protocol at POST7, and 5 of 7 completed the protocol at POST21. No comparison was carried out between ALT1 and POST21 due to the low number of subjects.

Resting Variables

The resting variables across acclimatization and reexposure have already been reported in detail elsewhere (54) and will not be reproduced in this paper.

Steady-State Method

Acclimatization. Compared with SL, the steady-state MCAv-CO_2 slope was elevated at ALT1 (by 79 ± 70%; $P < 0.001$), and was further elevated at ALT16 (by 89 ± 70% vs. ALT1; $P = 0.001$) (Table 1). Similarly, the steady-state MAP-CO_2 slope was elevated at ALT1 (by 0.24 ± 0.23 mmHg/mmHg; $P = 0.013$) and further elevated at ALT16 (by 0.80 ± 0.46 mmHg/mmHg vs. ALT1; $P < 0.001$). The steady-state CVCi-CO_2 slope was elevated at ALT1 (by 82 ± 79%; $P < 0.001$), and remained higher at ALT16 (by 93 ± 81%; $P < 0.001$ vs. SL, no difference with ALT1). V̇E at 40 mmHg was elevated at ALT1 compared with SL (by 14.8 ± 12.3 l/min; $P = 0.011$), and further elevated at ALT16 (by 48.3 ± 32.0 l/min vs. ALT1; $P < 0.001$).

Reexposure. Upon reexposure to altitude, it appears that the acclimatization gained in the steady-state MCAv-CO_2 slope was not retained at POST7 ($P = 0.145$ vs. ALT1). Compared with ALT16, the steady-state MCAv-CO_2 slope was lowered at both POST7 and POST21 ($P = 0.029$ and $P = 0.003$, respectively), but nevertheless remained higher compared with SL ($P < 0.001$ and $P = 0.024$, respectively). Similarly, 49% of the acclimatization gained in the MAP-CO_2 slope was retained at POST7. Specifically, the MAP-CO_2 slope remained higher at POST7 compared with ALT1 ($P = 0.005$). Compared with ALT16, the MAP-CO_2 slope was lowered at both POST7 and POST21 ($P < 0.001$ for both). Nevertheless, the MAP-CO_2 slope was higher at POST7 and POST21 compared with SL ($P < 0.001$ and $P = 0.020$, respectively). In contrast, no difference was observed in the CVCi-CO_2 slope at POST7 compared with ALT1 or ALT16 ($P = 0.980$ and $P = 0.804$, respectively), but it remained higher compared with SL ($P <$ 0.001). Likewise, the CVCi-CO_2 slope tended to remain higher

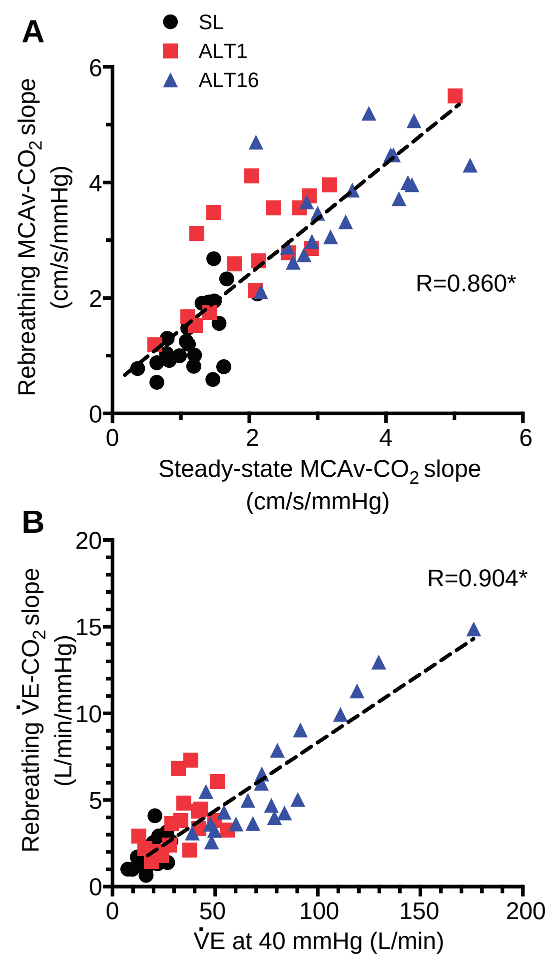

Fig. 3. Comparison of steady-state and rebreathing estimates of cerebrovascular and ventilatory responsiveness of CO_2 with acclimatization to 5,260 m. *Significant correlations ($P < 0.05$).

Table 1. *Cerebrovascular and ventilatory reactivity parameters during the steady-state and modified rebreathing*

	SL (n = 21)	ALT1 (n = 17)	ALT16 (n = 20)	POST7 (n = 14)	POST21 (n = 5)
Steady-state					
MCAv-Pa_{CO_2} slope ($cm \cdot s^{-1} \cdot mmHg^{-1}$)	1.19 ± 0.42	2.16 ± 1.05*	3.39 ± 0.89*†	2.68 ± 0.88*‡	2.06 ± 0.57*‡
CVCi-Pa_{CO_2} slope (%/mmHg)	3.35 ± 1.21	5.87 ± 2.60*	5.75 ± 1.85*	5.89 ± 1.23*	5.41 ± 1.78*
MAP-Pa_{CO_2} slope (l/min)	0.03 ± 0.24	0.28 ± 0.19*	1.06 ± 0.45*†	0.56 ± 0.29*‡	0.32 ± 0.18*‡
$\dot{V}_E$ at 40 mmHg (l/min)	19.15 ± 4.89	34.06 ± 12.23*	80.05 ± 32.32*†	49.03 ± 13.68*†‡	43.25 ± 7.56*‡
Modified rebreathing					
MCAv-$P_{ET_{CO_2}}$ slope ($cm \cdot s^{-1} \cdot mmHg^{-1}$)	1.34 ± 0.60	2.95 ± 1.11*	3.67 ± 0.87*†	—	—
$\dot{V}_E$-CO_2 slope ($l \cdot min^{-1} \cdot mmHg^{-1}$)	1.90 ± 0.81	3.49 ± 1.51*	6.28 ± 3.56*†	—	—
$\dot{V}_E$ recruitment threshold (mmHg)	38.7 ± 3.4	33.7 ± 3.7*	29.2 ± 2.1*†	—	—

All values are mean ± SD. SL, sea level; ALT1, day 1 at high altitude; ALT16, day 16 at high altitude; POST7, reexposure following 7 days at low altitude; POST21, reexposure following 21 days at low altitude. *Different from SL ($P < 0.05$); †different from ALT1 ($P < 0.05$); ‡different from ALT16 ($P < 0.05$).

at POST21 compared with SL ($P = 0.058$) but was not different from ALT16 ($P = 0.715$).

Upon reexposure, the effect of acclimatization on $\dot{V}_E$ at 40 mmHg was retained by 38% at POST7 ($P = 0.004$ vs. ALT1). Compared with ALT16, $\dot{V}_E$ at 40 mmHg was lower at POST7 and POST21 ($P = 0.001$ and $P < 0.001$, respectively), but these values remained higher compared with SL ($P < 0.001$ and $P = 0.001$, respectively).

Modified Rebreathing Method

Similar to the steady-state method, the rebreathing MCAv-CO_2 slope was elevated at ALT1 (by 137 ± 117%; $P < 0.001$), and further elevated at ALT16 (by 35 ± 33% vs. ALT1; $P = 0.040$) (Table 1). The rebreathing $\dot{V}_E$-CO_2 slope was elevated at ALT1 compared with SL (by 1.61 ± 1.14 $l \cdot min^{-1} \cdot mmHg^{-1}$; $P = 0.038$), and further elevated at ALT16 (by 2.86 ± 2.61 $l \cdot min^{-1} \cdot mmHg^{-1}$ vs. ALT1; $P = 0.004$). The ventilatory recruitment threshold was lowered at ALT1 (by 4.4 ± 4.0 mmHg; $P < 0.001$ vs. SL) and further lowered at ALT16 (by 4.4 ± 3.2 mmHg vs. ALT1; $P < 0.001$).

Acid-Base Buffering Capacity Correlations

Based on previous findings (16), we performed correlations between the pooled steady-state data with $[HCO_3^-]$ and found that resting $[HCO_3^-]$ correlated with the steady-state MCAv-CO_2 slope ($R = -0.771$) and $\dot{V}_E$ at 40 mmHg ($R = -0.723$; $P < 0.001$ for both) (Fig. 2).

Steady-State vs. Modified Rebreathing

We observed correlations between the steady-state and rebreathing MCAv-CO_2 slope at SL ($R = 0.609$; $P = 0.003$), ALT1 ($R = 0.817$; $P < 0.001$), and ALT16 ($R = 0.596$; $P = 0.007$), whereas the pooled MCAv-CO_2 slopes (combined SL, ALT1, and ALT16) between the two methods also correlated well ($R = 0.860$; $P < 0.001$) (Fig. 3). Likewise, there were significant correlations between $\dot{V}_E$ at 40 mmHg and the rebreathing $\dot{V}_E$-CO_2 slope at SL ($R = 0.476$; $P = 0.029$), ALT1 ($R = 0.506$; $P = 0.038$), and ALT16 ($R = 0.927$; $P < 0.001$), whereas the pooled ventilatory data across all time points also correlated ($R = 0.904$; $P < 0.001$).

DISCUSSION

The present study is the first to assess the effect of altitude acclimatization and reexposure on cerebrovascular CO_2 reactivity using both the steady-state and modified rebreathing methods. We demonstrate that cerebrovascular CO_2 reactivity was elevated immediately upon arrival at 5,260 m and is further elevated following 16 days of acclimatization regardless of the method of assessment. In addition, we found that cerebrovascular and ventilatory responsiveness to CO_2 remains elevated upon reexposure to altitude, despite 7 or 21 days at low altitude. Because these changes in cerebrovascular and ventilatory responsiveness to CO_2 correlated with the changes in resting arterial $[HCO_3^-]$ across all time points, we speculate that these changes might be partly due to an altered pH buffering capacity associated with exposure to high altitude. Our data thus demonstrate that the changes in cerebrovascular and ventilatory control gained due to altitude acclimatization over a period of 16 days are partially preserved upon subsequent exposure to altitude, at least for up to a period of 3 wk spent at low altitude.

Effects of Acclimatization on Cerebrovascular CO_2 Reactivity

Our findings extend those from Fan et al. (16, 17) by demonstrating that the MCAv-CO_2 slope is elevated upon arrival at 5,260 m and further elevated following 16 days of acclimatization (Fig. 1*A*). Importantly, previous studies by Fan et al. (16, 17) assessed MCAv-CO_2 slope in subjects who spent 8 days ascending to 5,050 m, whereas the subjects in the present study ascended rapidly to altitude (~3 h), thus making direct comparison difficult. Our findings contradict those of Lucas et al. (30), who found that the MCAv-CO_2 slope was initially elevated at 5,050 m, but had returned toward sea level values following 2 wk at 5,050 m. However, because $P_{ET_{O_2}}$ was not controlled, the MCAv-CO_2 slopes reported by Lucas et al. (30) reflect MCAv changes from polkilocapnic hypoxia (room air breathing at 5,050 m: $P_{ET_{O_2}}$ ~48 mmHg and $P_{ET_{CO_2}}$ 26–22 mmHg) to hypercapnic hyperoxia ($P_{ET_{O_2}} > 310$ mmHg and $P_{ET_{CO_2}}$ ~30 mmHg), and thus do not represent isolated reactivity to CO_2. Rupp et al. (49) recently found the MCAv response to steady-state hypoxic hypercapnia ($P_{ET_{O_2}} = 55$ mmHg) to be reduced following 5 days at 4,350 m. Therefore, discrepancies between findings by Rupp et al. (49) and those of the present study can be attributed the differences in $P_{ET_{O_2}}$ (55 mmHg vs. >200 mmHg), altitude (4,350 m vs. 5,260 m), and acclimatization state of the subjects (5 days vs. 16 days). The results from the present study demonstrate for the first time that cerebrovascular CO_2 reactivity per se is enhanced with acclimatization to high altitude when studied using a background level of hyperoxia. Furthermore, discrepancies between studies

highlight how methodological differences can yield vastly different results. Thus future studies are warranted to clarify the effect of hypoxic and hyperoxic background on assessing cerebrovascular functions at both sea level and following ascent to high altitude.

Altered Acid-Base Buffering Capacity?

During altitude acclimatization, there is a progressive and parallel reduction in arterial and cerebrospinal fluid (CSF) bicarbonate concentration, which serves to compensate for the changes in pH associated with hyperventilation-induced hypocapnia (12, 13, 20). These changes in acid-base buffering capacity, in both the arterial and CSF compartments, would lead to a greater rise in arterial and CSF $[H^+]$ for a given rise in Pa_{CO_2}. In support of this notion, lowering CSF bicarbonate concentration elevates the cerebrovascular CO_2 reactivity in an anesthetized dog model (27), whereas bicarbonate infusion increases cerebral perfusion pressure in patients with posttraumatic head injury (9), elevates cerebral blood volume in preterm infants (57), and lowers ventilation in healthy exercising humans at SL (44). As such, it has been suggested that the MCAv responses to CO_2 at high altitude are linked to changes in arterial acid-base balance (16, 25). In the present study, we observed concomitant increases in cerebrovascular and ventilatory responsiveness to CO_2 with acclimatization to high altitude and reexposure (Fig. 1), which occurred in parallel to the changes in $[HCO_3^-]$ (Fig. 2). While such correlations do not imply causality, the possible role for acid-base status changes on cerebrovascular and ventilatory responsiveness to CO_2 at high altitude remains to be further studied.

Interaction Between Cerebrovascular and Ventilatory Responsiveness to CO_2

Interaction between cerebrovascular CO_2 reactivity and central chemoreceptor activation was first alluded to by Heyman et al. (22) and has been subsequently expanded upon by others (10, 16–18, 38, 43, 60–62). It was postulated that changes in cerebrovascular CO_2 reactivity affect the stability of the ventilatory response to CO_2 by modulating the degree of H^+ washout at the level of the central chemoreceptor (38). Accordingly, a blunted cerebrovascular CO_2 reactivity would lead to less central H^+ washout and subsequently greater central chemoreceptor activation. Conversely, an enhanced cerebrovascular CO_2 reactivity would result in lower central $[H^+]$ and therefore lower ventilatory CO_2 sensitivity. In agreement with previous altitude studies (16, 17), we observed concomitant increases cerebrovascular and ventilatory responsiveness to CO_2 (Fig. 1). These findings seem to contradict the modulating role of cerebrovascular CO_2 reactivity on central chemoreceptor activation, possibly due to other overriding factors such as enhanced central chemosensitivity and changes in acid-base balance associated with ascent to high altitude. Future work is necessary to further unravel the interaction between the regulation of cerebral blood flow and ventilation.

Going Back Up

Despite the large body of literature regarding high-altitude acclimatization over the past century, the effect of prior exposure on physiological parameters during subsequent exposures is not well documented. Most attention has focused on the effect of a recent altitude exposure on the risk for AMS (7, 31, 45, 51) or the rate of ascent (56). However, the dose of previous altitude exposure and acclimatization were generally not controlled in these studies. Wu et al. (59) found a progressive reduction in the incidence of AMS, lower HR, and higher SpO_2 in lowland railroad workers over the course of several 7-mo exposures to high altitude interspersed with 5 mo spent at low altitude. Similarly, MacNutt et al. (32) found faster rate of ascent, lower AMS, and higher SpO_2 in trekkers with a recent altitude exposure compared with altitude-naive trekkers, despite a 7- to 30-day deacclimatization period. In the present study, we compared the cerebrovascular and ventilatory responsiveness to CO_2 with acclimatization and upon reexposure to 5,260 m following a period of either 7 or 21 days at low altitude. We found that 38% of the gain in ventilatory response to CO_2 over acclimatization was retained at POST7 (Fig. 1*C*), whereas essentially none of the gain in MCAv-CO_2 reactivity over acclimatization was retained at POST7 (Fig. 1*A*). Regardless of the underpinning mechanism(s), our findings suggest that the effect of previous altitude acclimatization over 16 days on the ventilatory response to CO_2 is partially retained after 7 days at low altitude, whereas it is reversed in the cerebrovascular response to CO_2. Our data extend findings by Muza et al. (36) showing that ventilatory acclimatization gained at 4,300 m is retained following 8 days spent at low altitude. Because we found the CVCi-CO_2 slope to be consistently elevated by 60–80% across all time points (Fig. 1*D*), whereas the changes in MAP-CO_2 slope closely follow the changes in MCAv-CO_2 slope (Fig. 1*B*), we speculate that the changes in MCAv-CO_2 slope at high altitude can be primarily accounted for by an enhanced sensitivity of the cerebral vessels to CO_2, whereas the remainder can be attributed to an enhanced perfusion pressure response.

Steady-State or Modified Rebreathing Method?

There has been much debate over the use of the steady-state or the modified rebreathing method for the assessment of cerebrovascular and ventilatory control, and attempts at consensus have produced no uniform agreement [(18, 40), also see (2, 14) for reviews]. The steady-state ventilatory responses to CO_2 were found to be either similar (34, 37, 40–42, 47) or lower (6, 18, 23, 55) compared with rebreathing estimates, whereas steady-state cerebrovascular CO_2 reactivity has been shown to be consistently higher than rebreathing values (18, 40–42). The present study demonstrates that the changes in cerebrovascular and ventilatory CO_2 responsiveness with altitude acclimatization were similar between the steady-state and the modified rebreathing method (Table 1), possibly due to tight control of arterial P_{CO_2} and P_{O_2} with our end-tidal clamping setup. Moreover, we observed strong correlations in these parameters between the two methods across all time points (Fig. 3). We therefore conclude that both methods can be used to assess the changes in cerebrovascular and ventilatory responses to CO_2 with high altitude exposure and acclimatization, provided that the level of CO_2 is comparable across all the conditions, under identical levels of background O_2.

Limitations

Although the present study provided the opportunity to assess the effects of acclimatization and reexposure to 5,260 m on cerebrovascular CO_2 reactivity, an important methodological consideration should be acknowledged when interpreting our findings. In the present study, transcranial Doppler ultrasound (TCD) was used to measure MCAv as an index of global CBF changes during initial exposure, acclimatization, and subsequent reexposure to 5,260 m. This is based on the assumption that *1*) the MCA carries approximately upward of 80% of the overall blood flow to the respective hemisphere (29); *2*) changes in MCAv reflect changes in global CBF (8, 52); *3*) the changes in MCAv in response to Pa_{CO_2} changes are comparable to the changes in internal carotid blood flow (50); and *4*) the diameter of the MCA does not change during the observed changes in arterial blood gases (52). In support, MCAv has been shown to reflect changes in CBF assessed with the direct Fick method, at least during initial exposure to high altitude (33, 35, 48).

Recent findings by Wilson et al. (58) indicate that the diameter of the MCA, as measured using TCD, varies depending on the altitude (e.g., 5.30 mm at 75 m, 5.51 mm at 3,500 m, 5.23 mm at 5,300 m, and 9.34 mm at 7,950 m). Importantly, the results reported by Wilson et al. (58) demonstrate that the MCA diameter remains relatively unchanged up to 5,300 m. It should be noted that the MCA diameters measured with TCD in that study were 80–90% greater than the values obtained using magnetic resonance imaging in the same subjects. Because our measurements were carried out in background hyperoxia ($P_{ET_{CO_2}}$ >300 mmHg), it seems unlikely that our cerebral blood velocity values would be confounded by any effect of hypoxia-induced vasodilation of the MCA. Further studies are needed to evaluate MCAv responses to CO_2 while holding $P_{ET_{O_2}}$ at consistent levels of hypoxia.

Conclusion

Findings from the present study clearly show that both cerebrovascular and ventilatory responsiveness to CO_2 is elevated upon arrival at high altitude and further elevated with acclimatization. We demonstrate for the first time that this effect of high-altitude acclimatization on the ventilatory response to CO_2 is partially retained after a period at low altitude, whereas prior acclimatization has no effect on the cerebrovascular response to CO_2. Our data suggest that the increased cerebrovascular CO_2 reactivity with acclimatization may be accounted for by the changes in acid-base balance in the blood and possibly the CSF compartment.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.-L.F., A.W.S., O.E., A.T.L., and R.C.R. conception and design of research; J.-L.F., A.W.S., O.E., and N.B. performed experiments; J.-L.F. and A.W.S. analyzed data; J.-L.F., A.W.S., N.B., B.K., and R.C.R. interpreted results of experiments; J.-L.F. prepared figures; J.-L.F. drafted manuscript; J.-L.F., A.W.S., O.E., B.K., A.T.L., and R.C.R. edited and revised manuscript; J.-L.F., A.W.S., O.E., N.B., B.K., A.T.L., and R.C.R. approved final version of manuscript.

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Research Paper

Experimental Physiology

AltitudeOmics: effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery

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New Findings

- **What is the central question of this study?**
  Hypoxia associated with ascent to high altitude may threaten cerebral oxygen delivery. We sought to determine whether there are regional changes in the distribution of cerebral blood flow that might favour oxygen delivery to areas associated with basic homeostatic functions to promote survival in this extreme environment.
- **What is the main finding and its importance?**
  We show evidence of a 'brain-sparing' effect during acute exposure to high altitude, in which there is a slight increase in relative oxygen delivery to the posterior cerebral circulation. This may serve to support basic regulatory functions associated with the brainstem and hypothalamus.

Cerebral hypoxaemia associated with rapid ascent to high altitude can be life threatening; yet, with proper acclimatization, cerebral function can be maintained well enough for humans to thrive. We investigated adjustments in global and regional cerebral oxygen delivery (D_{O_2}) as 21 healthy volunteers rapidly ascended and acclimatized to 5260 m. Ultrasound indices of cerebral blood flow in internal carotid and vertebral arteries were measured at sea level, upon arrival at 5260 m (ALT1; atmospheric pressure 409 mmHg) and after 16 days of acclimatization (ALT16). Cerebral D_{O_2} was calculated as the product of arterial oxygen content and flow in each respective artery and summed to estimate global cerebral blood flow. Vascular resistances were calculated as the quotient of mean arterial pressure and respective flows. Global cerebral blood flow increased by ∼70% upon arrival at ALT1 ($P < 0.001$) and returned to sea-level values at ALT16 as a result of changes in cerebral vascular resistance. A reciprocal pattern in arterial oxygen content maintained global cerebral D_{O_2} throughout acclimatization, although D_{O_2} to the posterior cerebral circulation was increased by ∼25% at ALT1 ($P = 0.032$). We conclude that cerebral D_{O_2} is well maintained upon acute exposure and acclimatization to hypoxia, particularly in the posterior and inferior regions of the brain associated with vital homeostatic functions. This tight regulation of cerebral D_{O_2} was achieved through integrated adjustments in local vascular resistances to alter cerebral perfusion during both acute and chronic exposure to hypoxia.

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Introduction

Although the brain represents only about 2% of body weight, it is a highly metabolic tissue that receives ∼15% of cardiac output and accounts for ∼20% of total body oxygen consumption at rest (Wade & Bishop, 1962). Maintenance of cerebral oxygen delivery (D_{O_2}) is essential for vital cerebral functions associated with homeostasis. In the face of severe hypoxaemia, such as experienced during rapid ascent to extreme altitudes (>8000 m), reduction in cerebral D_{O_2} results in loss of consciousness within seconds (Luft *et al.* 1951; Luft & Noell, 1956) and death within minutes (Bert, 1943). However, with staged acclimatization to progressively higher elevations, cerebral D_{O_2} can be maintained well enough for humans to reach the summit of Mount Everest (8848 m) without supplemental oxygen. The mechanisms responsible for this remarkable plasticity in cerebral D_{O_2} are complex and not completely understood.

Cerebral D_{O_2} is the product of cerebral blood flow (CBF) and arterial oxygen content (C_{aO_2}). It is well established that CBF rises upon acute exposure to high altitude and returns to near sea-level values with acclimatization (Severinghaus *et al.* 1966; Huang *et al.* 1987; Jensen *et al.* 1990), while C_{aO_2} decreases in acute hypoxia and returns to sea-level values with acclimatization. These opposing CBF and C_{aO_2} responses to altitude appear to offset one another and maintain cerebral D_{O_2} throughout acclimatization (Severinghaus *et al.* 1966; Wolff *et al.* 2002). The pattern of CBF change in response to hypoxia has been attributed to the relative balance of hypoxic vasodilatation and hypocapnic vasoconstriction in the brain (Xu & Lamanna, 2006; Brugniaux *et al.* 2007). During acute, severe hypoxia, vasodilatation typically exceeds vasoconstriction, resulting in greater CBF (Mardimae *et al.* 2012; Willie *et al.* 2012). With acclimatization, increased ventilatory drive reduces the arterial partial pressure of CO_2 (P_{aCO_2}) and improves the arterial partial pressure of O_2 (P_{aO_2}), tipping the balance in favour of vasoconstriction and restoring CBF to pre-exposure values. Changes in the P_{aO_2}/P_{aCO_2} ratio have been shown to account for ∼40% of the variation in global CBF over acclimatization (Lucas *et al.* 2011), with other biochemical (e.g. pH, HCO_3^-, nitric oxide) and haematological factors (e.g. haemoglobin, haematocrit, blood viscosity) presumably accounting for the rest of the response (Todd *et al.* 1994; Tomiyama *et al.* 1999; Severinghaus, 2001) to maintain global cerebral D_{O_2}.

Recent data demonstrate that acute normobaric hypoxia (i.e. breathing hypoxic gas) affects the regional distribution of CBF within the brain. Data from positron emission tomography (PET) studies show greater perfusion of the brainstem, hypothalamus, thalamus and cerebellum during acute hypoxia, with (Binks *et al.* 2008) or without controlled levels of P_{aCO_2} (Buck *et al.* 1998). Regional differences in cerebrovascular reactivity to O_2 and CO_2 have been postulated to control the distribution of CBF. Vascular Doppler studies of the major tributary vessels of the brain suggest that a greater percentage of blood flow may be directed towards the posterior cerebral circulation, including the brainstem, in response to controlled levels of hypoxia and hypocapnia (Sato *et al.* 2012). From a teleological perspective, this could help preserve vital homeostatic functions at the expense of higher cognitive processing; however, it is unclear whether regional distribution of CBF is affected in a similar manner in hypobaric hypoxia (i.e. high altitude) or if it changes with acclimatization, because not all studies report significant regional differences (Huang *et al.* 1987; Willie *et al.* 2012, 2013).

Despite the importance of O_2 supply for cerebral function, longitudinal studies of cerebral D_{O_2} at high altitude are sparse. In a secondary analysis of data from original study by Severinghaus *et al.* (1966) of CBF at high altitude, global cerebral D_{O_2} in four subjects appeared stable and in excess of oxygen demand after 6–12 h and 3–5 days, respectively, of exposure to 3810 m (Severinghaus, 2001; Wolff *et al.* 2002). Using similar methodology (Kety–Schmidt technique), no differences were found in global cerebral D_{O_2} measured after 5 weeks at 5260 m and return to sea level (Møller *et al.* 2002). Unfortunately, these studies were based on a limited number of observations, which makes it difficult to detect small differences, if they existed (type II error), and used methodology that can only measure global cerebral D_{O_2}. A more recent magnetic resonance imaging (MRI) study with a larger sample size reported a tendency towards elevation of cerebral D_{O_2} after subjects returned from 2 days at 3800 m (Smith *et al.* 2013), but no measurements of regional cerebral D_{O_2} were made. Based the limited data to date, it is uncertain whether global or regional cerebral D_{O_2} varies over time at high altitude.

In this study, we used vascular Doppler technology in conjunction with arterial blood sampling to allow us to quantify global and regional changes in CBF and cerebral D_{O_2} in the field as healthy people rapidly ascended and acclimatized to high altitude (5260 m). We tested the hypothesis that upon acute exposure cerebral D_{O_2} would be maintained to regions of the brain associated with homeostasis at the expense of other tissues, but that these changes would normalize with acclimatization.

Methods

Subject recruitment and screening

This study was conducted as part of the AltitudeOmics project, for which a detailed description of the protocol is published elsewhere (Subudhi *et al.* 2014). Briefly, following institutional ethics approval from

the Universities of Colorado and Oregon and the US Department of Defense Human Research Protection Office, young, healthy, sea-level residents were recruited from the greater Eugene, OR area (elevation 128 m). Potential subjects were screened to exclude anyone who was born or had lived at altitudes >1500 m for longer than 1 year or had travelled to altitudes >1000 m in the past 3 months. After obtaining written informed consent, physical examinations and the Army Physical Fitness Test (push ups, sit ups and 3.2 km run) were performed to verify health and fitness status.

Study overview

To evaluate effects of altitude acclimatization on cerebrovascular haemodynamics, subjects were studied on three occasions, as follows: (i) at sea level (SL, 130 m); (ii) upon acute exposure to 5260 m (ALT1); and (iii) after 16 days of acclimatization (ALT16). Specifically, ∼4 weeks following SL measurements in Eugene, OR, subjects were flown to La Paz, Bolivia. They spent two nights at low altitude (Coroico, Bolivia; 1525 m) before being driven to the Chacaltaya Research Station at 5260 m while breathing supplemental oxygen. Acute responses to high altitude were assessed 2–4 h after arrival and cessation of supplemental oxygen (ALT1). Subjects acclimatized to altitudes ranging from 3800 to 5260 m over the next 15 days, with a majority of the time (75%) spent at 5250 m. Measurements were repeated on ALT16.

Instrumentation

Subjects were studied in an upright, seated position with feet on the floor. Arterial blood pressure was monitored via a fluid-filled pressure transducer (Utah Medical, Salt Lake City, UT, USA) positioned at heart level and attached to a 22 gauge catheter in a radial artery. Blood flow velocity in the left middle cerebral artery ($MCA_{velocity}$) was measured by transcranial Doppler (2 MHz probe, Spencer Technologies, Seattle, WA, USA; affixed to a custom-made headset) at depths ranging from 43 to 54 mm. Signal quality was optimized, and an M-mode screen shot was recorded to facilitate subsequent probe placements. Arterial saturation was measured on the right side of the forehead by pulse oximetry (Nellcor N-200, Mansfield, MA, USA). Limb lead electrodes were used to measure ECG (ADInstruments BioAmp, Colorado Springs, CO, USA and Sonosite Micromaxx, Bothell, WA, USA). Metabolic variables, including expired ventilation and gas concentrations were assessed via breath-by-breath (Medgraphics PFX, St Paul, MN, USA and Vacumed UVM, Ventura, CA, USA) and mixing chamber systems (Oxigraf O_2cap, Mountain View, CA, USA), calibrated with the same 3 l syringe and known concentrations of O_2 and CO_2 prior to each test. Additionally, core temperature was monitored by telemetry pill (CorTemp HQInc., Palmetto, FL, USA). Analog data were sampled and recorded at 200 Hz (ADinstruments Powerlab 16/30, Colorado Springs, CO, USA).

Cerebral blood flow

After verification of signal quality, resting data were recorded for 10 min while subjects breathed room air. At 6 min, 2 ml of arterial blood was drawn anaerobically for blood gas analysis (described in section ‘Cerebral oxygen delivery’). During the last 4 min of the resting period, the diameter and blood flow velocities in the left internal carotid (ICA; 1.5 cm distal to the carotid bifurcation) and vertebral arteries (VA; between spinous processes of C4 and C5) were recorded over a minimum of five cardiac cycles by a registered diagnostic sonographer (SonoSite Micromaxx L25 probe, Bothell, WA, USA). Briefly, vessel diameter from a longitudinal view was identified and measured with digital callipers in synchronization with the ECG trace to identify systole and diastole. Velocity was measured in the centre of the vessel with an insonation angle <60 deg and a sample volume maximized for vessel diameter. The peak velocity trace across cardiac cycles was used for calculation of mean velocity (time-averaged peak) and volumetric flow. This procedure was used to verify accurate tracing of the spectral envelop during data collection and results in higher values than the time-averaged mean method (Schöning *et al.* 1994). All data were downloaded in DICOM format for verification of measurements offline (Sante DICOM Editor, Athens, Greece).

Regional blood flow (in millilitres per minute) in the ICA and VA (ICA_{flow} and VA_{flow}) was determined using standard, validated ultrasound techniques (Hoskins, 2008), where:

$$\begin{aligned} \text{flow} = \pi \times (\text{diameter in centimetres}/2)^2 \\ \times \text{time averaged peak velocity in} \\ \text{centimetres per second} \times 60\ \text{s} \end{aligned}$$

Average coefficients of variation determined from three repeated measurements of ICA and VA flow measurements in seven subjects at SL were 4.0 ± 2.6 and 4.0 ± 2.1%, respectively.

Global CBF (gCBF) was estimated assuming symmetrical bilateral flow in the major tributary arteries of the brain (Ogoh *et al.* 2013; Willie *et al.* 2013) as follows:

$$\text{gCBF} = (ICA_{flow} + VA_{flow}) \times 2$$

Regional and global measurements of CBF were also expressed relative to estimates of cardiac output ($\%\dot{Q}$) derived from simultaneous intra-arterial blood pressure

Table 1. Cardiopulmonary and haematological values

Variable	Units	SL	ALT1	ALT16
Ventilation	$\mathrm{l\ min^{-1}}$	12.05 ± 2.50 (21)	11.93 ± 2.92 (17)	14.88 ± 2.65 (21)∗†
Arterial P_{O_2}	mmHg	102.2 ± 5.5 (21)	36.1 ± 2.8 (18)∗	45.3 ± 3.2 (20)∗†
Arterial P_{CO_2}	mmHg	38.1 ± 4.4 (21)	26.5 ± 3.1 (18)∗	20.9 ± 2.5 (20)∗†
Arterial O_2 saturation	%	98 ± 1 (21)	76 ± 6 (18)∗	82 ± 3 (20)∗†
Haemoglobin concentration	$\mathrm{g\ dl^{-1}}$	13.9 ± 1.4 (21)	14.2 ± 1.5 (18)∗	16.0 ± 2.0 (20)∗†
Arterial O_2 content	$\mathrm{ml\ dl^{-1}}$	19.4 ± 1.9 (21)	15.2 ± 2.1 (18)∗	18.4 ± 2.4 (20)∗†
Heart rate	$\mathrm{beats\ min^{-1}}$	76 ± 12 (21)	90 ± 16 (16)∗	96 ± 13 (20)∗
Stroke volume	ml	91 ± 27 (21)	85 ± 20 (16)	83 ± 21 (20)
Mean arterial blood pressure	mmHg	79 ± 8 (21)	76 ± 13 (16)	80 ± 10 (20)

Values are given as means ± SD (*n*). ∗Different at sea level (SL), and on the 1st and 16th days at 5260 m (ALT1, ALT16, respectively). †Different from ALT1.

traces (Bogert *et al.* 2010). Cerebral vascular resistance index (CVRi) was calculated as follows:

$$\mathrm{CVRi} = \mathrm{mean\ ABP/flow}$$

Cerebral oxygen delivery

Arterial blood was immediately analysed for P_{aO_2}, P_{aCO_2} (Siemens RAPIDLab 248, Erlangen, Germany), haemoglobin concentration ([Hb]), arterial oxygen saturation (S_{aO_2}; Radiometer OSM3, Copenhagen, Denmark) and haematocrit (M24 Centrifuge, LW Scientific, Lawrenceville, GA, USA). Blood gases were temperature corrected (Kelman & Nunn, 1966; Severinghaus, 1966). The C_{aO_2} (vol%) was calculated as follows:

$$C_{aO_2} = 1.39 \times [\mathrm{Hb}] \times S_{aO_2} + P_{aO_2} \times 0.003$$

Regional and global cerebral D_{O_2} were calculated as the products of C_{aO_2} and $\mathrm{ICA_{flow}}$, $\mathrm{VA_{flow}}$ and gCBF.

Data analysis

After verification of normality, mixed repeated-measures ANOVAs were used to analyse the interaction of time by sex for each variable of interest ($\alpha = 0.05$). Subsequent estimation-maximization and multiple-imputation (five trials) analyses verified negligible effects of missing values (SPSS 20, IBM, Chicago, IL, USA). Student's paired *t* tests (without imputation of missing values) were used for *post hoc* comparisons with the Holm procedure to control for type I error. *A priori* power calculations ($\alpha = 0.05$, $\beta = 0.20$) were integrated into the study design to limit type II error. Pearson product–moment correlations were used to describe shared variance between variables. Data are presented as means ± SD.

Based on the hypothesis that increased CBF may play a role in the pathogenesis of acute mountain sickness (AMS; Jensen *et al.* 1990; Baumgartner *et al.* 1994, 1999), a secondary analysis was performed to evaluate potential relationships (Spearman correlations) between changes in CBF and D_{O_2} with the severity of Lake Louise Questionnaire symptom scores reported in these subjects on ALT1 (Subudhi *et al.* 2014). Student's paired *t* tests were used to evaluate differences in CBF and D_{O_2} between those with severe AMS (Lake Louise Questionnaire symptoms scores ≥ 6, including headache) and those remaining healthy.

Results

Subject characteristics

Detailed baseline characteristics of the 21 subjects (12 men and nine women; 21 ± 1 years old) participating in AltitudeOmics are presented elsewhere (Subudhi *et al.* In Review). Men exhibited higher [Hb], C_{aO_2} and D_{O_2} than females over the course of the study (all $P < 0.05$), but as no interactions in CBF or D_{O_2} were detected throughout acclimatization, combined data are presented below.

Cerebral blood flow and oxygen delivery

Acute exposure to 5260 m (atmospheric pressure 408 ± 1 mmHg) decreased P_{aO_2}, S_{aO_2} and C_{aO_2} by 66.1 ± 5.4 mmHg, 22 ± 6% and 4.1 ± 1.2 ml $\mathrm{dl^{-1}}$, respectively (all $P < 0.001$; Table 1). This severe degree of hypoxia increased heart rate by 14 ± 11 beats $\mathrm{min^{-1}}$ ($P < 0.001$) without affecting mean arterial blood pressure ($P = 0.380$). Cerebral blood flow increased by 74 ± 81% in the ICA ($P = 0.018$), 59 ± 54% in the VA ($P = 0.001$) and 69 ± 57% globally ($P = 0.003$). Respective CVRi values fell (all $P < 0.001$; Table 2), allowing a larger percentage of cardiac output to perfuse the brain ($P = 0.010$). Increased $\mathrm{ICA_{flow}}$ was characterized by increased ICA velocity ($P = 0.004$) without a change in diameter ($P = 0.068$), while increased $\mathrm{VA_{flow}}$ was explained by an increase in VA diameter ($P = 0.005$) without a change in velocity ($P = 0.120$). The $\mathrm{MCA_{velocity}}$ was unchanged ($P = 0.953$). Increased gCBF offset the decrease in C_{aO_2} to maintain global cerebral D_{O_2} (Fig. 1), although a small increase in VA D_{O_2} was observed ($P = 0.039$; Fig. 2). Observed changes

Table 2. Cerebrovascular values

Variable	Units	SL	ALT1	ALT16
ICA diameter	cm	0.51 ± 0.08 (21)	0.54 ± 0.07 (16)	0.50 ± 0.07 (20)†
ICA velocity	cm s^{-1}	29.8 ± 8.2 (21)	38.9 ± 8.1 (16)*	32.1 ± 5.4 (20)†
ICA flow	ml min^{-1}	384 ± 197 (21)	556 ± 203 (16)*	379 ± 97(20)†
ICA CVRi	mmHg ml^{-1} min^{-1}	0.25 ± 0.12 (21)	0.16 ± 0.09 (16)*	0.23 ± 0.07 (19)†
VA diameter	cm	0.36 ± 0.06 (20)	0.41 ± 0.06 (16)*	0.36 ± 0.06 (19)†
VA velocity	cm s^{-1}	21.4 ± 4.4 (20)	24.4 ± 6.4 (16)	19.3 ± 7.1 (19)†
VA flow	ml min^{-1}	133 ± 47 (20)	206 ± 98 (16)*	122 ± 55 (19)†
VA CVRi	mmHg ml^{-1} min^{-1}	0.66 ± 0.24 (20)	0.46 ± 0.28 (16)*	0.84 ± 0.58 (19)†
gCBF	ml min^{-1}	1057 ± 413 (20)	1524 ± 456 (16)*	981 ± 223 (19)†
gCBF CVRi	mmHg ml^{-1} min^{-1}	0.09 ± 0.03 (20)	0.05 ± 0.02 (16)*	0.08 ± 0.02 (19)†
D_{O_2} ICA	ml min^{-1}	75 ± 37 (21)	84 ± 32 (16)	68 ± 19 (19)†
D_{O_2} VA	ml min^{-1}	26 ± 10 (20)	31 ± 16 (16)*	22 ± 11 (19)†
D_{O_2} gCBF	ml min^{-1}	206 ± 79 (20)	230 ± 74 (16)	181 ± 51 (19)†
MCA velocity	cm s^{-1}	59.5 ± 10.3 (21)	61.1 ± 13.3 (17)	57.7 ± 7.1 (21)
MCA CVRi	mmHg cm^{-1} s^{-1}	1.36 ± 0.25 (21)	1.28 ± 0.32 (17)	1.41 ± 0.24 (20)
ICA%$\dot{Q}$	%	5.4 ± 2.7 (21)	7.6 ± 2.7 (15)*	4.8 ± 1.4 (18)†
VA%$\dot{Q}$	%	1.9 ± 0.8 (20)	2.6 ± 1.1 (15)*	1.5 ± 0.7 (18)†
gCBF%$\dot{Q}$	%	15.0 ± 5.8 (20)	20.4 ± 6.2 (15)*	12.6 ± 3.4 (18)†

Values are given as means ± SD (*n*). *Different at sea level (SL), and on the 1st and 16th days at 5260 m (ALT1, ALT16, respectively). †Different from ALT1. Abbreviations: ICA, internal carotid artery; CVRi, cerebrovascular resistance index; VA, vertebral artery; gCBF, global cerebral blood flow; D_{O2}, oxygen delivery; MCA, middle cerebral artery; and Q, cardiac output.

in measures of regional and global CBF and D_{O_2} were not correlated with Lake Louise Questionnaire symptom scores of AMS ($r = -0.07$ to -0.23, $P = 0.38$–0.78), nor were they different between those reporting severe AMS and those remaining healthy ($P = 0.57$–0.97).

Following acclimatization, a 32 ± 36% rise in ventilation was accompanied by a 5.5 ± 2.7 mmHg decrease in P_{aCO_2} and 9.2 ± 4.1 mmHg increase in P_{aO_2} (ALT1 *versus* ALT16; all $P < 0.001$). The values of S_{aO_2} and [Hb] rose by 6 ± 5% and 1.8 ± 0.9 g dl^{-1}, respectively, improving C_{aO_2} by 3.1 ± 1.2 ml dl^{-1} (all $P < 0.001$; Table 1). Arterial blood pressure was unaffected by acclimatization (ALT1 *versus* ALT16; $P = 0.211$). The ICA$_{flow}$, VA$_{flow}$ and gCBF returned to SL values (SL *versus* ALT16; $P = 0.810$, 0.977 and 0.620, respectively; Table 2). Respective CVRi values increased as both ICA and VA diameters decreased from ALT1 to ALT16 (all $P < 0.020$) and restored the relative distribution of cardiac output back to SL values (SL *versus* ALT16; $P = 0.121$). Cerebral D_{O_2} fell from ALT1 to ALT16 (ICA D_{O_2} $P = 0.028$, VA D_{O_2} $P = 0.020$ and global D_{O_2} $P = 0.011$) as the reductions in CBF outweighed the increase in C_{aO_2} (Fig. 1); however, neither global nor regional cerebral D_{O_2} values fell below that measured at SL (all $P > 0.420$; Figs 1 and 2).

Discussion

This is the first study to assess regional cerebral oxygen delivery in the field over a period of acclimatization to high altitude. Our findings confirm that global cerebral D_{O_2} was preserved across acclimatization through a changing balance between CBF and C_{aO_2}, but there was a slight increase in relative D_{O_2} to the posterior cerebral circulation during acute exposure. Although changes in CBF and D_{O_2} were not associated with the incidence or severity of AMS, regional regulation of CBF may serve to support vital homeostatic cerebral functions in hypoxia.

Preservation of cerebral oxygen delivery

The increase in CBF upon arrival at high altitude and decrease back to sea-level values with acclimatization was opposed by changes in C_{aO_2} (Fig. 1). These responses preserved cerebral D_{O_2} close to sea-level values and affirm that components of C_{aO_2} (P_{aO_2}, S_{aO_2} and [Hb]) outweigh the influence of P_{aCO_2} in regulating CBF in severe hypoxia. Increased CBF upon arrival at high altitude resulted from reduced cerebral vascular resistance rather than increased blood pressure (Tables 1 and 2). Although a reduction in cerebral vascular resistance is commonly attributed to dilatation of pial and parenchymal arterioles in the brain (Fog, 1938), we observed an increased diameter of larger tributary arteries, supporting a global vascular response to this degree of hypoxia (Heistad *et al.* 1978; Faraci & Heistad, 1990; Willie *et al.* 2012). The mechanisms governing hypoxic vasodilatation are complex, involving local (e.g. astrocyte regulation, nitric oxide) and diffuse mechanisms (e.g. central chemoreception, autonomic nervous system), but all stem from a reduction in P_{aO_2} (Severinghaus, 2001; Xu & Lamanna, 2006). When P_{aO_2} is above 60 mmHg, little vasodilatation is evident (Mardimae *et al.* 2012; Willie *et al.* 2012). Below this threshold, the degree of vasodilatation increases exponentially and outweighs the degree of hypocapnic vasoconstriction

(Mardimae *et al.* 2012; Willie *et al.* 2012); presumably, to provide greater blood flow in a time of need. While the correlation between changes in gCBF and C_{aO_2} was not significant, the change in C_{aO_2} from SL to ALT1 was similar among all subjects and may not have afforded an appropriate range of values to detect the relationship that has previously been shown with progressive haemodilution (Korosue & Heros, 1992). Qualitatively, the ~70% increase in gCBF was within the expected range during acute hypocapnic hypoxia (Severinghaus, 1966, 2001; Jensen *et al.* 1990; Brugniaux *et al.* 2007) and proportional to the ~60% reduction in P_{aO_2} that was responsible for the reduction in C_{aO_2}. This reciprocal relationship, whether evolved or serendipitous, is advantageous for survival in these extreme conditions because it mitigates the negative consequences of cerebral hypoxaemia.

Although increased CBF has been suggested to play a role in the pathogenesis of AMS (Baumgartner *et al.* 1994), our results were more similar to those refuting the hypothesis (Jensen *et al.* 1990; Baumgartner *et al.* 1999). Regional and global CBF and D_{O_2} measurements were not correlated with AMS symptom scores and did not differentiate between those with severe AMS and those who remained healthy after rapid ascent to high altitude. Nonetheless, our data should be interpreted with caution because it is possible that increased CBF contributes to the development of AMS when other, yet to be described, factors are present.

Increased P_{aO_2} and decreased P_{aCO_2} after 16 days at high altitude are hallmarks of ventilatory acclimatization that are addressed elsewhere (Fan *et al.* 2014). As a result, P_{aO_2}-mediated vasodilatation was reduced and P_{aCO_2}-mediated vasoconstriction was increased, thereby lowering CBF. Assuming a cerebral O_2 reactivity of 3% CBF $\%S_{aO_2}^{-1}$ and a CO_2 reactivity of 4% CBF (mmHg $CO_2)^{-2}$ from a previous duplex ultrasound study (Willie *et al.* 2012), we could account for the entire decrease in gCBF across acclimatization. Specifically, the 5% increase in S_{aO_2} could be expected to reduce CBF by ~15% and the 5.5 mmHg decrease in P_{aCO_2} could be

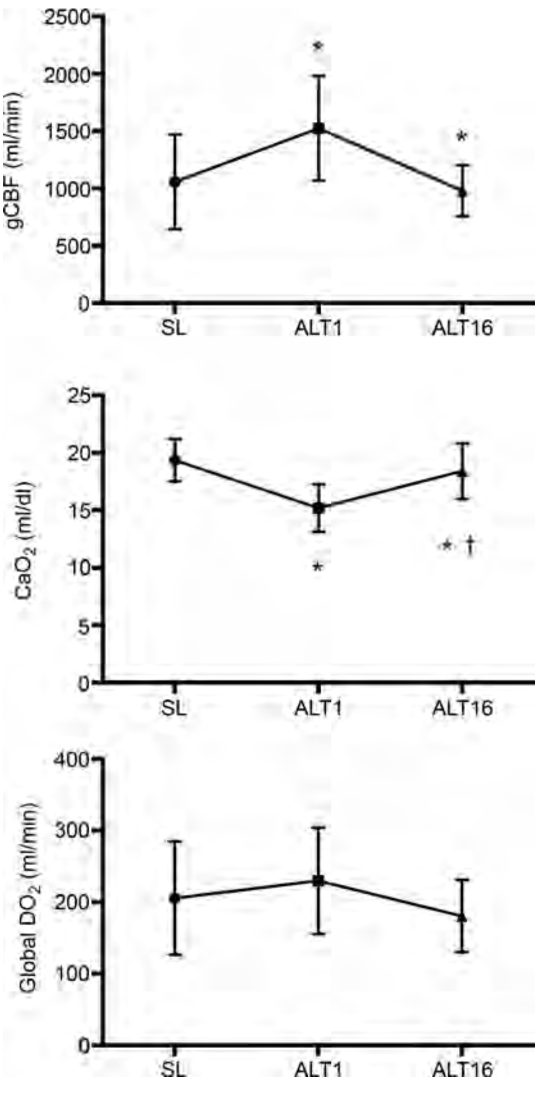

Figure 1. Reciprocal changes in global cerebral blood flow (gCBF) and arterial oxygen content (C_{aO_2}) maintained global cerebral oxygen delivery (D_{O_2}) throughout the study
∗Different from sea level (SL). †Different from arrival at altitude (ALT1). Abbreviation: ALT16, 16th day at 5260 m.

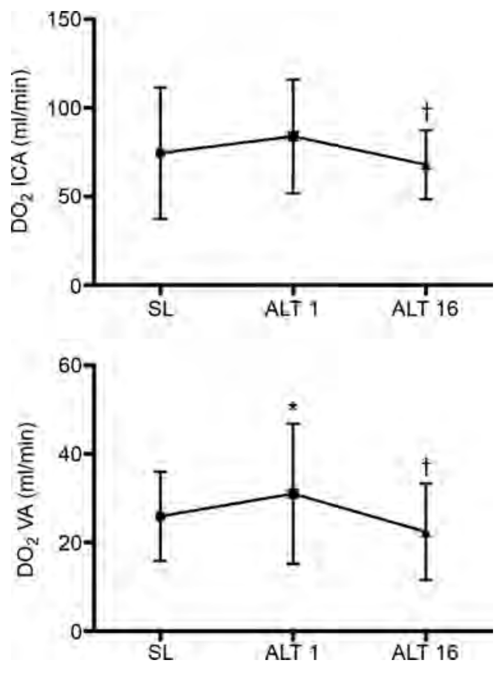

Figure 2. Regional oxygen delivery (D_{O_2}) increases in the vertebral artery (VA) but not in the internal carotid artery (ICA) on arrival at altitude (ALT1)
Regional D_{O_2} is reduced with acclimatization, but not below sea-level (SL) values. ∗Different from SL. †Different from ALT1.

expected to reduce CBF by ∼22%, thus accounting for the 36% decrease in gCBF we observed from ALT1 to ALT16 (Table 2). We acknowledge that increased cerebrovascular CO_2 reactivity with acclimatization in our subjects (Fan *et al.* 2014) may account for an even greater proportion of the net effect on CBF at ALT16. Also, the relative influence of other haematological factors, such as increased haematocrit and blood viscosity (Sorensen *et al.* 1974; Todd *et al.* 1994; Tomiyama *et al.* 1999) from erythropoiesis and plasma volume contraction, may have contributed to the reduction of CBF throughout acclimatization (data to be presented elsewhere). Yet our data suggest that the inherent vascular reactivities to O_2 and CO_2 are sufficient to maintain tight control over cerebral D_{O_2} in hypoxia. Consistent delivery of oxygen may help to offset the decreased P_{O_2} gradient (plasma to mitochondria) and support the cerebral metabolic demand for oxygen at this altitude (Severinghaus *et al.* 1966; Møller *et al.* 2002) to preserve cerebral function. Together, our data demonstrate that integrated mechanisms controlling cerebral blood flow are well suited to preserve global cerebral oxygen delivery at 5260 m.

Regional cerebral oxygen delivery

We observed a small increase in D_{O_2} through the posterior cerebral circulation upon arrival at high altitude (Table 2) that dissipated with acclimatization. The acute increase in D_{O_2} was characterized by an increase in VA diameter and supports recent findings of greater VA (*versus* ICA) vasoreactivity during acute hypoxia (Willie *et al.* 2012; Ogoh *et al.* 2013). Of note, Ogoh *et al.* (2013), showed that acute hypoxia (∼15 min) increased VA, but not ICA, blood flow. Given that the areas perfused by the VA include the brainstem and posterior aspects of the thalamus and hypothalamus, increased blood flow and D_{O_2} to these regions during acute hypoxia (Buck *et al.* 1998; Binks *et al.* 2008) may be seen as necessary to maintain vital homeostatic functions (Sheldon *et al.* 1979; Bilger & Nehlig, 1993). As increased cardiorespiratory drive with acclimatization was not associated with a continued elevation of VA D_{O_2}, we speculate that the increased VA D_{O_2} during acute hypoxia was protective, to defend against a potential threat to oxygen supply, rather than merely to support neuronal metabolic activity associated with heightened autonomic activity (i.e. neurovascular coupling). Although such hypothetical explanations for regional differences in the regulation of CBF and D_{O_2} are intriguing, our results must be interpreted with caution because measured differences were small and are not consistently reported in the literature (Huang *et al.* 1987; Willie *et al.* 2013). Future studies with more focal measurements of D_{O_2} (e.g. PET and MRI) and neuronal activity in key regulatory regions of the brain, as well as measurements of neurovascular coupling (as an index of neuronal plasticity) during acute and prolonged hypoxia are needed to yield further insight into this question.

Brain sparing

Reduced cerebral vascular resistance associated with vasodilatation upon arrival at altitude can explain the proportional increase in CBF and greater allocation of cardiac output. This effect could be magnified if there is net constriction in other vascular beds at rest. Previous studies have shown that superior mesenteric and renal artery blood flow decrease in acute hypoxia and could allow for greater perfusion of the brain (Greene & Roach, 2004). With acclimatization, cerebral vascular resistance and blood flow returned to sea-level values. These results are similar to fetal 'brain-sparing' effects (Campbell *et al.* 1967; Peeters *et al.* 1979; Sheldon *et al.* 1979) that are presumed to preserve vital homeostasis during hypoxia *in utero* (Pearce, 2006; Salihagić-Kadić *et al.* 2006). Similar effects have also been shown in newborn dogs (Cavazzuti & Duffy, 1982), piglets (Goplerud *et al.* 1989) and premature infants (Daven *et al.* 1983). The largest response to hypoxia tends to occur in the brainstem during the early postnatal period and decreases with age (Bilger & Nehlig, 1993). We are the first to demonstrate that such a brain-sparing reaction exists in healthy human adults exposed to acute hypoxia and recedes with acclimatization. Preferential distribution of cardiac output to the brain upon acute altitude exposure may represent a conserved mechanism that protects against hypoxic brain damage in mammals, particularly in brain regions associated with basic cardiovascular and respiratory control during periods of acute hypoxia. Measurements of regional cerebral metabolism are needed to determine whether brain sparing effectively matches D_{O_2}, or if the increase in CBF represents a protective form of overcompensation.

Limitations

Our rapid ascent profile in combination with supplemental oxygen during transport from low to high altitude was designed to induce an abrupt change in P_{aO_2}, similar to that which can be achieved in laboratory studies with hypoxic gas or hypobaric chambers. As such, our results must be interpreted in this context and thus may be expected to be different from other field studies that have followed more traditional progressive ascents (Huang *et al.* 1987; Jensen *et al.* 1990; Baumgartner *et al.* 1994; Willie *et al.* 2013).

We used duplex sonography primarily because it is a non-invasive technique that can be used in field settings. This technique yields volumetric measurements, in terms

of millilitres per minute, which, based on first principles, can be multiplied by C_{aO_2} to yield D_{O_2}. Our low coefficients of variation were in line with a previous study showing similarity between duplex sonography and both PET and xenon inhalation methods of measuring gCBF (Schöning & Scheel, 1996). Nevertheless, we acknowledge that all these techniques are limited by the lack of an absolute standard for validating CBF. Our gCBF measurements were based on unilateral, left-sided measurements of the ICA and VA, which are the main arteries perfusing the brain. While left VA flow has been reported to be ∼20% higher than the right (Schöning *et al.* 1994), this was not expected to have an effect on global measurements because ICA flow represents the majority of gCBF (Schöning & Scheel, 1996). Yet, unilateral VA measurements may have influenced our finding of increased VA D_{O_2}. Future studies are needed to determine whether brain-sparing effects are attenuated when independent measurements of left and right VA flow are summed.

Given that the ICA feeds the MCA, we expected that changes in ICA flow would be reflected in $MCA_{velocity}$. This was not the case; ICA flow increased by ∼70% while $MCA_{velocity}$ was unchanged throughout the study. A similar discrepancy between ICA flow and $MCA_{velocity}$ has been described previously by Willie *et al.* (2012) and argued to support dilatation of the MCA in hypoxia (Wilson *et al.* 2011). We calculated that a 12% increase in MCA diameter could explain the measured discrepancy between ICA_{flow} and $MCA_{velocity}$. This exact degree of vasodilatation has recently been demonstrated at high altitude with a colour-coded ultrasound technique (Willie *et al.* 2013), yet because additional studies are needed to clarify artery-specific responses to hypoxia and validate MCA-diameter measurement techniques, we chose to refrain from further interpretation of $MCA_{velocity}$.

Summary and implications

Overall, our findings highlight the integrative nature of responses that preserve oxygen delivery to the brain at high altitude. Regional cerebral vasoreactivity to O_2 and CO_2 may favour oxygen delivery to posterior and inferior regions of the brain during acute hypoxia to sustain vital cerebral functions associated with homeostasis. Whether these mechanisms evolved to promote survival in conditions provoking cerebral hypoxia is not clear at present, but further research in this area may yield important insights into human tolerance and adaptation to chronic states of hypoxaemia.

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Additional Information

Competing interests

None declared.

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AltitudeOmics: exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude

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Abstract

Aims: We asked whether acclimatization to chronic hypoxia (CH) attenuates the level of supraspinal fatigue that is observed after locomotor exercise in acute hypoxia (AH).

Methods: Seven recreationally active participants performed identical bouts of constant-load cycling (131 ± 39 W, 10.1 ± 1.4 min) on three occasions: (i) in normoxia (N, P_IO_2, 147.1 mmHg); (ii) in AH (F_IO_2, 0.105; P_IO_2, 73.8 mmHg); and (iii) after 14 days in CH (5260 m; P_IO_2, 75.7 mmHg). Throughout trials, prefrontal-cortex tissue oxygenation and middle cerebral artery blood velocity (MCAV) were assessed using near-infrared-spectroscopy and transcranial Doppler sonography. Pre- and post-exercise twitch responses to femoral nerve stimulation and transcranial magnetic stimulation were obtained to assess neuromuscular and corticospinal function.

Results: In AH, prefrontal oxygenation declined at rest (Δ7 ± 5%) and end-exercise (Δ26 ± 13%) ($P < 0.01$); the degree of deoxygenation in AH was greater than N and CH ($P < 0.05$). The cerebral O_2 delivery index (MCAV × C_aO_2) was 19 ± 14% lower during the final minute of exercise in AH compared to N ($P = 0.013$) and 20 ± 12% lower compared to CH ($P = 0.040$). Maximum voluntary and potentiated twitch force were decreased below baseline after exercise in AH and CH, but not N. Cortical voluntary activation decreased below baseline after exercise in AH (Δ11%, $P = 0.014$), but not CH (Δ6%, $P = 0.174$) or N (Δ4%, $P = 0.298$). A twofold greater increase in motor-evoked potential amplitude was evident after exercise in CH compared to AH and N.

Conclusion: These data indicate that exacerbated supraspinal fatigue after exercise in AH is attenuated after 14 days of acclimatization to altitude. The reduced development of supraspinal fatigue in CH may have been attributable to increased corticospinal excitability, consequent to an increased cerebral O_2 delivery.

Keywords adaptation, altitude, exercise, transcranial magnetic stimulation.

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The mechanisms underpinning impairments in exercise performance in hypoxia are not fully understood, but multiple peripheral and central mechanisms of fatigue have been proposed (Nybo & Rasmussen 2007, Amann & Calbet 2008, Perrey & Rupp 2009). The rate of development of peripheral fatigue is increased during intense locomotor exercise in acute hypoxia (Amann *et al.* 2006b, Goodall *et al.* 2012). This has been documented in numerous human studies as an increased decline in the force response to motor nerve stimulation after exercise and an increased rate of rise in electromyogram (EMG) signals during exercise (Amann & Calbet 2008). Amann *et al.* (2006a) suggested that the accelerated development of peripheral fatigue and associated intramuscular metabolic changes in acute moderate hypoxia restricts central motor drive preventing excessive end-exercise locomotor muscle fatigue under conditions of attenuated arterial oxygenation. It was subsequently demonstrated that in acute severe hypoxia, peripheral fatigue becomes the less important variable and the primary limitation to exercise transfers to a hypoxia-sensitive central component of fatigue (Amann *et al.* 2007). Less is known about the mechanism(s) of fatigue during locomotor exercise in chronic hypoxia. We recently reported the accelerated development of peripheral fatigue after locomotor exercise in acute hypoxia to be similar after a period of acclimatization (14 days) to high altitude; conversely, the level of central fatigue was attenuated (Amann *et al.* 2013). The measure of central fatigue, however, was determined using peripheral stimulation, and the responsiveness of the brain to muscle pathway after a period of chronic hypoxia remains unknown.

Transcranial magnetic stimulation (TMS) has been used to specify the site of fatigue within the central nervous system in acute severe hypoxia (Goodall *et al.* 2010, 2012). When TMS is delivered over the motor cortex during a maximal voluntary contraction (MVC), it is possible to detect a twitch-like increment in force in the active muscle. That is, despite maximal effort, motor cortical output at the time of stimulation is insufficient to drive the motor neurones maximally. An increase in this increment in force after exercise provides evidence of a reduced cortical voluntary activation, indicative of supraspinal fatigue (Gandevia *et al.* 1996, Todd *et al.* 2003). Further, EMG recordings in response to cortical stimuli (motor-evoked potential [MEP]) can be monitored to assess changes in excitability of the brain to muscle pathway. Descending volleys evoked from cortical stimulation depend on the stimulus intensity and excitability of corticospinal cells, whereas responses in the muscle depend on transmission through relevant excitatory and inhibitory interneurones and excitability of the motor neurone pool (Taylor & Gandevia 2001). Hypoxia affects neuronal function *in vitro* (Nieber *et al.* 1999); however, acute hypoxia appears to have negligible effects on resting MEPs elicited by TMS (Szubski *et al.* 2006, Goodall *et al.* 2010, Rupp *et al.* 2012). A MEP evoked during muscular contraction is followed by an interval of EMG silence, the so-called cortical silent period (CSP). The initial phase of the CSP has been attributed to inhibitory spinal mechanisms (Inghilleri *et al.* 1993), whereas the later period (>100 ms) represents increased cortical inhibition (Inghilleri *et al.* 1993, Chen *et al.* 1999, Taylor & Gandevia 2001). Szubski *et al.* (2006) found a shorter CSP in acute hypoxia, suggestive of a reduced corticospinal inhibition during the exercise.

Responsiveness of the corticospinal pathway and the associated development of central fatigue after locomotor exercise during periods of prolonged hypoxia have not been studied. A recent investigation found an increase in corticospinal excitability (increased resting MEP) after a period of prolonged acute hypoxia (Rupp *et al.* 2012); however, the mechanisms for this response and the associated effects upon the development of central fatigue during locomotor exercise have not been studied. We have recently related the development of supraspinal fatigue during exercise in severe acute hypoxia to a reduction in cerebral O_2 availability (Goodall *et al.* 2012). Acclimatization to altitude not only brings about improvements in arterial oxygenation but also improvements in cerebrovascular function (Ainslie & Ogoh 2009, Lucas *et al.* 2011). It is unknown how haematologic (e.g., haemodynamic and cerebrovascular) adaptations might serve to impact corticospinal excitability and the development of supraspinal fatigue during locomotor exercise in chronic hypoxia. Accordingly, the aim of the present study was to assess corticospinal excitability and supraspinal fatigue after locomotor exercise in chronic hypoxia. We hypothesized that altered cerebrovascular and corticospinal responses after a period of acclimatization to high altitude would reduce the severity of supraspinal fatigue compared to that observed in acute hypoxia.

Methods

Ethical approval

All procedures conformed to the Declaration of Helsinki and were approved by the Universities of Colorado Denver, Oregon and Utah Institutional Review Boards and the US Department of Defense Human Research Protection Office.

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Participants

This study was conducted as part of the AltitudeOmics project examining the integrative physiology of human responses to hypoxia (Subudhi *et al.* 2014). After written informed consent, seven (five male) recreationally active sea level habitants participated in the study (mean ± SD age, 21 ± 1 year; stature, 1.78 ± 0.10 m; body mass, 69 ± 11 kg; maximum O_2 uptake [$\dot{V}o_{2max}$], 46.4 ± 8.2 mL kg^{-1} min^{-1} [participant IDs: 1,2,3,5,6, 7,10]). The participants were non-smokers, free from cardiorespiratory disease, born and raised at <1500 m, and had not travelled to elevations >1000 m in the 3 months prior to investigation. Participants arrived at the laboratory in a rested and fully hydrated state, at least 3 h post-prandial, and avoided strenuous exercise in the 48 h preceding each trial. They also refrained from caffeine for 12 h before each test, while alcohol and prophylactic altitude medication were prohibited for the entire duration of the investigation. All of the subjects participated in a companion study investigating the acclimatization-induced effects on peripheral measures of neuromuscular fatigue (Amann *et al.* 2013); while the data were obtained from the same protocol described below, the primary TMS and cerebral oxygenation-related outcome measures in the current study do no overlap with previous analyses.

Experimental design

Participants completed a preliminary trial and three experimental trials. Each trial was conducted at the same time of day and separated by at least 5 days during a 12-week period. During the preliminary trial, participants were thoroughly familiarized with the methods used to assess neuromuscular function and performed a maximal incremental exercise test in normoxia for the determination of $\dot{V}o_{2max}$ and peak workload (W_{peak}); further maximal incremental tests were performed in AH and CH (Subudhi *et al.* 2014). During the experimental trials, participants performed constant-load exercise at a workload equal to 50% W_{peak} obtained in the preliminary trial: (i) to the limit of tolerance in acute normobaric hypoxia (AH: F_IO_2 = 0.105; Eugene, Oregon, barometric pressure [BP] = 750 ± 2 mmHg; P_IO_2 = 73.8 ± 0.2 mmHg); (ii) for the same absolute intensity and duration as in trial 1, but in normoxia (N: Eugene, Oregon, BP = 750 ± 2 mmHg; P_IO_2 =147.1 ± 0.5 mmHg); and (iii) for the same absolute intensity and duration as in trial 1, but after 14 days at 5260 m above sea level (CH: Mt. Chacaltaya, Bolivia, BP = 409 ± 1 mmHg; P_IO_2 = 75.7 ± 0.1 mmHg). Participants were flown to La Paz, Bolivia, where they spent two nights at low altitude (Coroico, 1525 m), before being driven to the Chacaltaya Research Station at 5260 m. Before and within 2.5 min after each exercise trial, twitch responses to supramaximal femoral nerve stimulation and TMS were obtained to assess fatigue. During AH, the post-exercise measurements were made while participants continued to breathe the hypoxic gas. Cerebrovascular, cardiorespiratory and perceptual responses, as well as EMG activity of the vastus lateralis (VL), were assessed throughout each trial.

Force and EMG recordings

Knee extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Tedea, Basingstoke, UK). The load cell was fixed to a custom-built chair and connected to a non-compliant cuff attached around the participant's right leg just superior to the right ankle. Participants sat upright in the chair with the hips and knees at 90° of flexion. EMG activity was recorded from the VL and biceps femoris (BF). Surface electrodes were placed 2 cm apart over the muscle bellies, and a reference electrode was placed over the patella. The electrodes were used to record the compound muscle action potential (M-wave) elicited by electrical stimulation of the femoral nerve and the MEP elicited by TMS. Signals were amplified (gain 1000; Force: custom-built bridge amplifier; EMG: PowerLab 26T, ADInstruments Inc, Oxfordshire, UK), band-pass filtered (EMG only: 20–2000 Hz), digitized (4 kHz; PowerLab 26T, ADInstruments Inc), acquired and later analysed (LabChart v7.0, ADInstruments Inc).

Neuromuscular function

Force and EMG variables were assessed before and immediately after each exercise trial. Prior to each trial, MVC force was determined from three, 3 s contractions. Femoral nerve stimulation was delivered at rest approximately 2 s after the MVC to determine the potentiated quadriceps twitch force ($Q_{tw,pot}$). TMS was delivered during brief (approx. 5 s) maximal and submaximal voluntary contractions for the determination of cortical voluntary activation. Each set of contractions comprised 100, 75 and 50% MVC efforts separated by approximately 5 s of rest. The contraction sets were repeated three times, with 15 s between each set. Visual feedback of the target force was provided via a computer monitor.

Femoral nerve stimulation

Single electrical stimuli (200 μs) were delivered to the right femoral nerve via surface electrodes (CF3200, Nidd Valley Medical Ltd, North Yorkshire, UK) via a

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constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The cathode was positioned over the nerve high in the femoral triangle; the anode was placed midway between the greater trochanter and the iliac crest. The site of stimulation that produced the largest resting twitch amplitude and M-wave (M_{max}) was located. Single stimuli were delivered beginning at 100 mA and increasing by 20 mA until plateaus occurred in twitch amplitude and M_{max}. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current 253 ± 60 mA).

Transcranial magnetic stimulation

TMS was delivered via a concave double cone coil (110 mm diameter; maximum output 1.4 T) powered by a mono-pulse magnetic stimulator (Magstim 200, The Magstim Company Ltd, Whitland, UK). The coil, placed over the vertex, preferentially stimulated the left hemisphere (postero-anterior intracranial current flow) and was held in the optimal position to elicit a large MEP in the VL and a small MEP in the antagonist (BF). This optimal coil position was marked on the scalp with indelible ink to ensure reproducibility of the stimulation. Resting motor threshold (rMT) was determined at the beginning of each experimental trial. Briefly, TMS was first delivered with the coil placed over the optimal site of stimulation at a subthreshold intensity of 35% maximum stimulator output. Stimulus intensity was then increased in 5% steps until consistent motor-evoked potentials (MEPs) with peak-to-peak amplitudes of more than 50 μV were evoked. Thereafter, stimulus intensity was reduced in 1% steps until an intensity was reached that elicited an MEP of at least 50 μV in 5 of 10 trials (Groppa *et al.* 2012). The stimulation intensity that elicited rMT was increased by 30%; thus, the experimental stimulation intensity was 130% of rMT. This stimulation intensity elicited a large MEP in the VL (area between 60 and 100% of M_{max} during knee extensor contractions ≥50% MVC; Fig. 1), indicating the TMS stimulus activated a high proportion of knee extensor motor units, while causing only a small MEP in the BF (amplitude <20% of MEP during knee extensor contractions).

Constant-load exercise

Participants sat on an electromagnetically braked cycle ergometer (Velotron Dynafit Pro, Racermate, Seattle, WA) while baseline cardiorespiratory and cerebrovascular data were collected for 3 min. The participants warmed-up for 5 min at 10% W_{peak} (26 ± 8 W) before the workload was increased to 50% normoxic

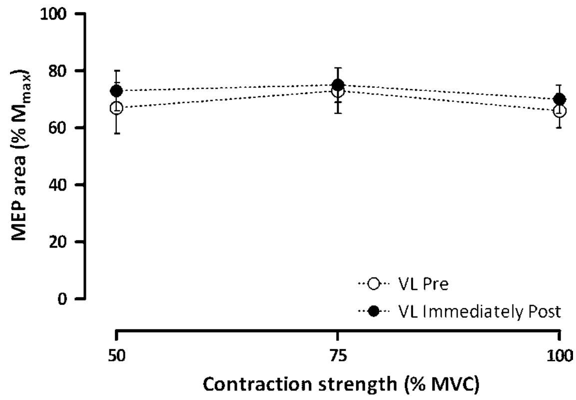

Figure 1 Mean area of motor-evoked potentials (MEP) recorded from the vastus lateralis (VL) in response to stimulation over the motor cortex during varying contraction intensities pre- (○) and post-exercise (●) (mean for all conditions). The TMS responses were compared to the area of the maximal M-wave (M_{max}) evoked by peripheral stimulation of the femoral nerve. Data are means ± SE for 7 participants.

W_{peak} (131 ± 39 W). This intensity was chosen to maximize the tolerable duration of exercise in the hypoxic conditions. The participants remained seated throughout exercise and maintained a target pedal cadence equivalent to that chosen during the incremental exercise test (88 ± 3 rpm). Task failure was reached when cadence dropped below 60% of the target rpm for >5 s. Constant-load exercise was performed firstly in AH; the achieved time (10.1 ± 1.4 min) was then replicated in N and CH.

Tissue oxygenation and cerebrovascular responses

Cerebral oxygenation was assessed using a multi-channel NIRS instrument (Oxymon III, Artinis) (Subudhi *et al.* 2009, 2011). Changes in oxygenated, deoxygenated and total cerebral haemoconcentrations (μM) were expressed relative to the resting baseline recorded in each experimental condition. Arterial oxygen saturation was estimated using forehead pulse oximetry (S_pO_2; Model N-595, Nellcor, Pleasonton, CA). Excellent agreement between the pulse oximeter and arterial O_2 saturation across the range of values in the present study has been published (Romer *et al.* 2007). Haemoglobin concentration [Hb] was measured (OSM-3, Radiometer, Copenhagen, Denmark) in resting arterial blood samples. Samples were collected during the primary physiological protocols at sea level (2–4 days prior to the first exercise trial in the present study) and on the 16th day at 5260 m (2 days following the constant-load exercise trial in the present study) (Subudhi *et al.* under review at PLoSOne). Arterial O_2 content (C_aO_2) was estimated

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using the equation: ([Hb] × 1.39 × S_pO_2/100). Resting [Hb] in combination with the measured S_pO_2 during the exercise protocol were used to obtain C_ao_2 throughout exercise in all conditions. Blood velocity in the left middle cerebral artery (MCAV) was determined using transcranial Doppler (Spencer Technologies, Seattle, WA). The custom-made NIRS headset was modified to hold a 2 MHz probe positioned over the left temporal window. Measurements were optimized at an average penetration depth of 50 ± 3 mm. An index of cerebral O_2 delivery was calculated as the product of MCAV and C_aO_2. It was assumed that changes in MCAV would reflect changes in cerebral blood flow based on evidence that the middle cerebral artery diameter changes minimally in response to hypoxia and hypocapnia (Poulin & Robbins 1996).

Cardiorespiratory and perceptual responses

Ventilatory and pulmonary gas exchange indices were assessed using an online system (in AH & N Medical Graphics PFX, St. Paul, MN, USA; & in CH Oxigraf O_2cap, Mountain View, CA, USA). Heart rate was identified from the peak MCAV envelopes. Ratings of perceived exertion for dyspnoea and limb discomfort were obtained using the CR10 scale at baseline and every minute throughout exercise (Borg 1982). In CH, symptoms of acute mountain sickness were assessed on the day of a trial using the Lake Louise Score (Roach *et al.* 1993).

Data analysis

Cortical voluntary activation was assessed by measuring the force responses to motor cortex stimulation during submaximal and maximal contractions. Corticospinal excitability increases during voluntary contraction (Rothwell *et al.* 1991); thus, we estimated the amplitude of the resting twitch evoked by TMS (ERT; Goodall *et al.* 2009, Sidhu *et al.* 2009a). Cortical voluntary activation (%) was subsequently quantified using the equation: (1 – [SIT/ERT] × 100).

The peak-to-peak amplitude and area of evoked MEPs and M_{max} were measured offline. To ensure the motor cortex stimulus activated a high proportion of the knee extensor motor units, the area of vastus lateralis MEP was normalized to that of M_{max} elicited during the MVC at the beginning of each trial (Taylor *et al.* 1999) (Fig. 1). The duration of the CSP evoked by TMS during MVC was quantified as the duration from stimulation to the continuous resumption of post-stimulus EMG exceeding ± 2 SD of pre-stimulus EMG (>50 ms prior to stimulus). VL EMG signals during exercise were rectified and smoothed (15 ms), then quantified as the mean integrated area during each cycle revolution and averaged over each minute of exercise. A computer algorithm identified the onset and offset of activity where the rectified EMG signals deviated >2 SD from baseline for >100 ms.

Reliability coefficients

On a separate day, the responses to TMS, femoral nerve stimulation and MVC were repeated twice in all participants. The two assessment procedures were separated by a 2 min walk followed by 5 min of rest. Coefficient of variation (CV) and intraclass correlation coefficient (ICC) were calculated to evaluate test–retest reliability. All correlations were statistically significant and indicated, in combination with the CVs, a high level of reproducibility: cortical voluntary activation, CV = 1.4%, ICC = 0.82; CSP, CV = 7.1%, ICC = 0.93; ERT, CV = 10.2%, ICC = 0.84; MEP/M_{max}, CV = 9.6%, ICC = 0.66; M_{max}, CV = 11.4%, ICC = 0.98; 100% MVC/MEP, CV = 14.1%, ICC = 0.96; 75% MVC/MEP, CV = 10.2%, ICC = 0.98; 50% MVC/MEP, CV = 7.2%, ICC = 0.99; MVC, CV = 4.7%, ICC = 0.94; and $Q_{tw,pot}$, CV = 4.8%, ICC = 0.97.

Statistical analysis

Data are presented as means ± SD in the text and means ± SE in the figures. A 3 × 2 repeated measures ANOVA on condition (3 [AH, N, CH]) and time (2 [pre, post]) was used to test for within-group differences. When ANOVA revealed significant interactions, post hoc comparisons were made using the least significant differences test. Statistical significance was set at $P < 0.05$. All analyses were conducted using SPSS (v19, IBM Corporation, New York, USA).

Results

Exercise responses

The exercise workload was 131 ± 39 W (50% N W_{peak}), which equated to 83% W_{peak} in AH and 74% W_{peak} in CH. Cerebral oxygenation data are shown in Figure 2. During N, oxyhaemoglobin was unchanged from baseline to warm-up and total haemoglobin was increased during the final minute of exercise ($P = 0.658$ and 0.007 respectively). During AH, deoxygenated haemoglobin increased from baseline to warm-up ($P = 0.006$); this response was exaggerated towards end-exercise ($P < 0.001$). During CH, deoxygenated haemoglobin increased at end-exercise ($P = 0.015$) in line with increased total haemoglobin ($P = 0.043$). Overall, these results demonstrate that the degree of cerebral deoxygenation (Δ deoxygenated

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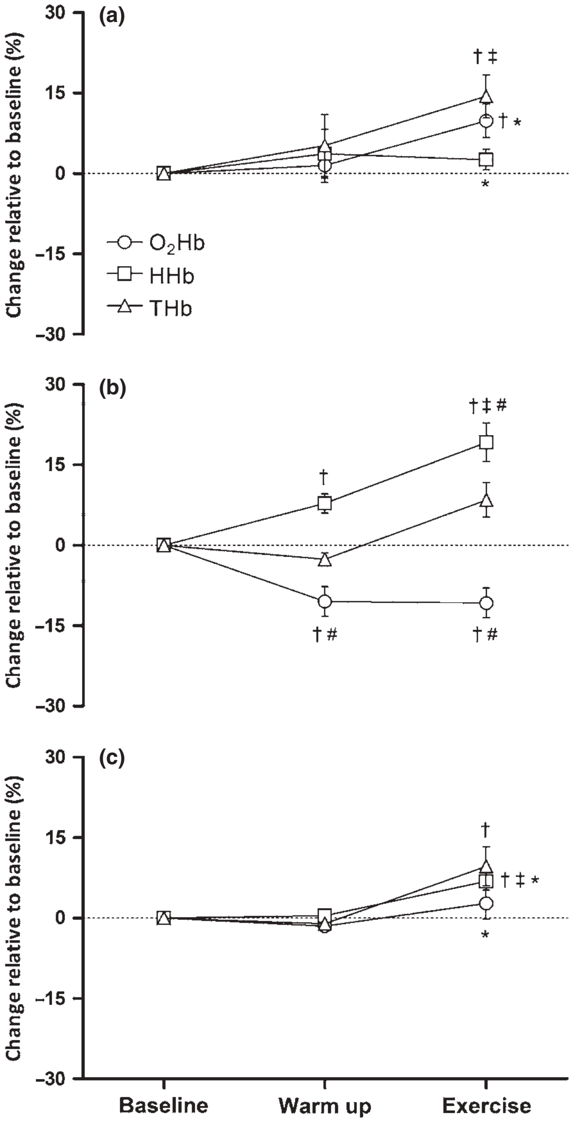

Figure 2 Cerebral oxygenation at resting baseline, during the final 30 s of a 3 min warm-up (28 W) and during the final 30 s of constant-load exercise (131 W) in normoxia (N; panel a), acute hypoxia (AH; panel b) and chronic hypoxia (CH; panel c). Data are means ± SE for 7 participants. †$P < 0.05$ versus respective baseline; ‡$P < 0.05$ versus respective warm-up; *$P < 0.05$ versus AH; #$P < 0.05$ versus CH. Resting baseline in AH denotes the value after 10 min wash in of the hypoxic gas. O_2Hb, oxygenated haemoglobin; HHb, deoxygenated haemoglobin; and THb, total haemoglobin.

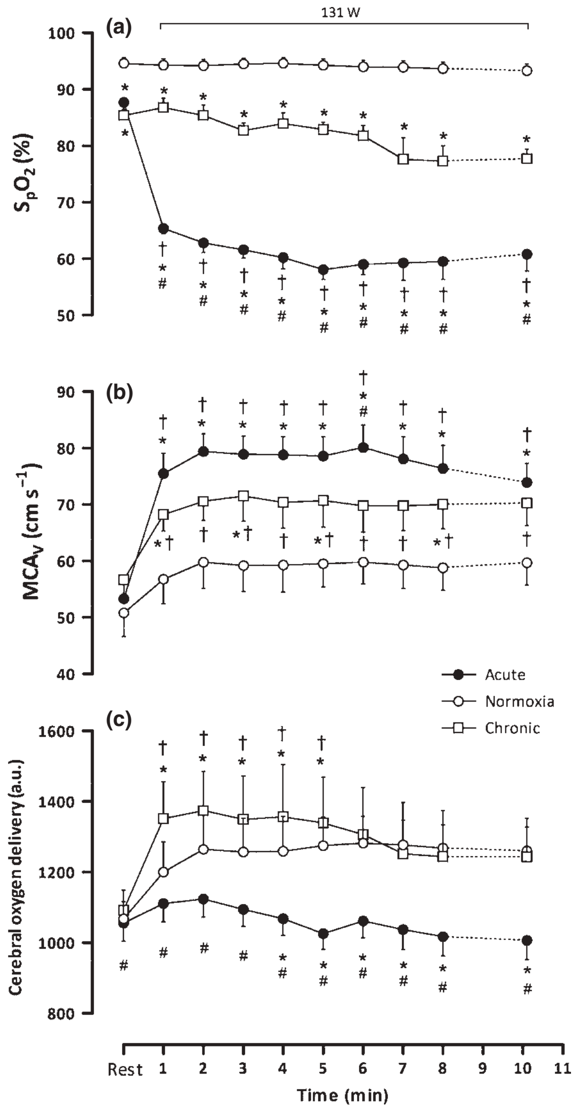

Figure 3 Arterial oxygen saturation (S_pO_2) (a), cerebral blood flow velocity (MCAV) (b) and middle cerebral artery O_2 delivery (c) index (MCAV × C_aO_2) during constant-load exercise (131 W) in normoxia (N), acute hypoxia (AH) and chronic hypoxia (CH). Values are plotted for the duration of the shortest trial (8 min) and extrapolated to the group mean exercise time (10.1 min). Data are means ± SE for 7 participants. †$P < 0.05$ versus rest; *$P < 0.05$ versus N; #$P < 0.05$ versus CH.

haemoglobin) in AH was greater than that observed in N and CH ($P < 0.05$).

S_pO_2 and MCAV data are shown in Figure 3. Acute exposure to hypoxia decreased S_pO_2 at rest (Δ7 ± 4%; $P = 0.009$) and during the final minute of exercise (Δ34 ± 10%; $P < 0.001$). Resting S_pO_2 in CH was 85 ± 2% ($P < 0.001$ vs. N; $P = 0.330$ vs. AH), and in the final minute of exercise had fallen to 78 ± 5% ($P < 0.001$ vs. N; $P = 0.002$ vs. AH). No changes in S_pO_2 were apparent in N ($P > 0.702$). Resting MCAV did not differ between conditions at baseline (pooled average, 54 ± 9 cm s^{-1}; $P = 0.544$). MCAV did not increase from rest at any time point in N ($P > 0.108$). MCAV increased from rest to the final minute of exercise in AH (40 ± 15%; $P < 0.001$) and CH (25 ± 14%; $P = 0.016$), but did not differ between conditions (Fig. 3).

Haemoglobin concentration was 1.42 ± 0.03 g L^{-1} in N and 1.63 ± 0.31 g L^{-1} in CH ($P = 0.005$).

Resting P_aO_2 was reduced in AH compared to N (39.1 ± 4.8 vs. 103.3 ± 8.7 mmHg, $P < 0.001$), was increased in CH relative to AH (58.8 ± 3.2 mmHg, $P < 0.001$), but was still lower than N ($P < 0.001$). C_aO_2 was lower at rest in AH versus N (19.8 ± 1.9 vs. 21.5 ± 2.9 mL dL^{-1}; $P = 0.013$); during the final minute of exercise C_aO_2 in AH was 36 ± 8% lower than N ($P < 0.001$) and 22 ± 9% lower than in CH ($P = 0.001$). C_aO_2 was lower at rest in CH versus N (19.4 ± 2.6 vs. 21.5 ± 2.9 mL dL^{-1}; $P < 0.001$) and during the final minute of exercise (17.6 ± 2.9 vs. 21.2 ± 2.9 mL dL^{-1}; $P = 0.725$). Consequently, cerebral O_2 delivery index (MCAV × C_aO_2) was 19 ± 14% lower during the final minute of exercise in AH compared to N ($P = 0.013$) and 20 ± 12% lower compared to CH ($P = 0.040$). No differences were evident between N and CH at rest ($P = 0.783$) or during the final minute of exercise ($P = 0.797$) (Fig. 3).

Cardiorespiratory data are shown in Table 1. Respiratory frequency and minute ventilation ($\dot{V}_E$) rose substantially over time in all conditions; $\dot{V}_E/\dot{V}CO_2$ during the final minute of exercise in AH and CH was approximately twofold greater than in N ($P < 0.001$); $\dot{V}_E/\dot{V}CO_2$ during the final minute of exercise was 28% higher in CH compared to AH ($P < 0.001$). During the final minute of exercise, whole body $\dot{V}o_2$ was not different across the three conditions ($P = 0.411$). Dyspnoea and limb discomfort at end-exercise were higher in AH compared to N ($P < 0.001$ and $P = 0.048$, respectively), but were not different compared to CH ($P = 0.714$ and 0.549 respectively). Integrated EMG activity at end-exercise was higher in AH compared to N (32%; $P = 0.029$), but not CH (16%; $P = 0.303$). There were no reported symptoms of acute mountain sickness during CH.

Pre- and post-exercise responses

Peripheral and central measures of excitability are shown in Table 2.

Neuromuscular responses. MVC did not differ between conditions at baseline (AH, 392 ± 77 N; N, 386 ± 90 N; CH, 376 ± 39 N; $P = 0.942$). MVC was reduced post-exercise in AH (339 ± 77 N, $P = 0.011$) and CH (346 ± 93 N, $P = 0.032$), but not N (387 ± 87 N, $P = 0.684$). The reductions in MVC

Table 1 Cardiorespiratory and perceptual responses at rest and during the final minute of constant-load cycling (131 W) in normoxia, acute hypoxia and chronic hypoxia

		Normoxia	Acute hypoxia	Chronic hypoxia
HR (beats min^{-1})	Rest	81 ± 7†	90 ± 9	104 ± 16
	Final min	150 ± 16*	173 ± 14	167 ± 16
$\dot{V}_E$ (l min^{-1})	Rest	14.3 ± 2.4	20.0 ± 2.6	24.5 ± 5.4
	Final min	60.0 ± 9.6*†	108.8 ± 24.7†	128.5 ± 30.0
f_R (breaths min^{-1})	Rest	15.6 ± 3.6	17.5 ± 4.5	13.0 ± 3.4
	Final min	31.4 ± 4.9*†	51.6 ± 8.7†	54.8.6 ± 9.9
V_T (l)	Rest	1.07 ± 0.37	1.30 ± 0.34	1.47 ± 0.63
	Final min	2.00 ± 0.45	2.07 ± 0.43	2.41 ± 0.58
$\dot{V}O_2$ (l min^{-1})	Rest	0.49 ± 0.10	0.45 ± 0.08	0.45 ± 0.12
	Final Min	2.45 ± 0.51	2.34 ± 0.58	2.07 ± 0.50
$\dot{V}CO_2$ (l min^{-1})	Rest	0.44 ± 0.09	0.55 ± 0.09	0.39 ± 0.08
	Final min	2.32 ± 0.51	2.69 ± 0.62†	1.94 ± 0.50
$\dot{V}_E/\dot{V}O_2$	Rest	30.7 ± 2.7*†	47.4 ± 6.5†	55.9 ± 14.9
	Final min	25.2 ± 2.4*†	51.2 ± 15.0†	62.9 ± 9.2
$\dot{V}_E/\dot{V}CO_2$	Rest	33.9 ± 2.7†	37.9 ± 6.5†	63.4 ± 6.8
	Final min	26.2 ± 2.6*†	41.7 ± 6.9†	67.1 ± 9.1
RPE, dyspnoea	Rest	7.0 ± 0.0	7.3 ± 0.5	7.1 ± 0.4
	Final min	11.4 ± 2.4*†	19.4 ± 0.8	19.1 ± 10.7
RPE, limb	Rest	7.1 ± 0.4	7.1 ± 0.4	7.0 ± 0.0
	Final min	12.3 ± 3.3*	19.9 ± 0.4	17.6 ± 11.7

HR, heart rate; $\dot{V}_E$, minute ventilation; f_R, respiratory frequency; V_T, tidal volume;$\dot{V}O_2$, oxygen uptake; ;$\dot{V}CO_2$, carbon dioxide output; RPE, ratings of perceived exertion.

Values are means ± SD for 7 participants. Resting values were measured during the 5th minute of breathing the test gas mixture.

*$P < 0.05$ vs. acute hypoxia.

$^{†}P < 0.05$ vs. chronic hypoxia.

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Table 2 Peripheral and central measures of excitability assessed before and after constant-load cycling (131 W) in normoxia, acute hypoxia and chronic hypoxia

		Normoxia	Acute hypoxia	Chronic hypoxia
Rest				
M_{max} amplitude (mV)	Pre	$6.9 \pm 2.0^{\dagger}$	$8.6 \pm 3.7^{\dagger}$	14.9 ± 8.3
	Post	6.7 ± 1.7	9.0 ± 4.1	14.0 ± 8.2
MEP amplitude (mV)	Pre	$0.19 \pm 0.12^{\dagger}$	$0.19 \pm 0.11^{\dagger}$	0.41 ± 0.28
	Post	0.11 ± 0.06	0.11 ± 0.10	$0.21 \pm 0.18^{\#}$
MEP/M_{max} (%)	Pre	2.6 ± 1.3	2.7 ± 1.9	4.1 ± 4.2
	Post	1.8 ± 1.2	1.5 ± 1.3	2.6 ± 3.4
Within contraction				
M_{max} amplitude 100% (mV)	Pre	8.9 ± 1.7	9.9 ± 3.2	13.0 ± 6.1
	Post	9.0 ± 1.9	10.0 ± 3.3	11.9 ± 5.4
MEP amplitude 100% (mV)	Pre	3.8 ± 1.5	$3.1 \pm 1.0^{\dagger}$	7.1 ± 4.7
	Post	4.0 ± 2.7	3.2 ± 1.0	6.5 ± 4.4
MEP amplitude 75% (mV)	Pre	$3.9 \pm 1.5^{\dagger}$	$2.9 \pm 1.4^{\dagger}$	7.6 ± 4.9
	Post	4.3 ± 2.6	$3.3 \pm 1.2^{\dagger}$	6.9 ± 3.9
MEP amplitude 50% (mV)	Pre	$2.54 \pm 0.87^{\dagger}$	$2.16 \pm 0.52^{\dagger}$	6.5 ± 4.8
	Post	$2.99 \pm 2.01^{\dagger}$	$2.56 \pm 0.95^{\dagger}$	6.4 ± 4.5
MEP/M_{max} (%) 100% MVC	Pre	35 ± 17	33 ± 14	52 ± 17
	Post	39 ± 20	37 ± 15	52 ± 19
MEP/M_{max} (%) 75% MVC	Pre	40 ± 15	$34 \pm 19^{\dagger}$	58 ± 18
	Post	42 ± 17	$38 \pm 18^{\dagger}$	57 ± 13
MEP/M_{max} (%) 50% MVC	Pre	$28 \pm 14^{\dagger}$	$26 \pm 10^{\dagger}$	50 ± 21
	Post	$30 \pm 15^{\dagger}$	$31 \pm 17^{\dagger}$	54 ± 23
CSP (ms)	Pre	198 ± 58	174 ± 46	186 ± 36
	Post	188 ± 64	171 ± 35	196 ± 51

M_{max}, maximal motor response; MEP, motor evoked potential; CSP, cortical silent period.
Values are means ± SD for 7 participants.
$^{\dagger}P < 0.05$ vs. chronic hypoxia.
$^{\#}P < 0.05$ vs. Pre.

were not different between conditions ($P \geq 0.119$). $Q_{tw,pot}$ did not differ between conditions at baseline (AH, 107 ± 13 N; N, 105 ± 12 N; CH, 110 ± 16 N; $P = 0.752$). $Q_{tw,pot}$ was reduced post-exercise in AH (84 ± 14 N, $P = 0.005$) and CH (90 ± 18 N, $P = 0.011$), but not N (102 ± 12 N, $P = 0.692$). On average, resting M_{max} in CH displayed a twofold increase compared to AH and N ($P < 0.019$); however, the change in M_{max} during MVC was not statistically significant ($P > 0.058$). Neither measure of M_{max} changed pre- to post-exercise in any condition ($P \geq 0.610$). Pooled across conditions, pre-exercise ERT (mean $r^2 = 0.95$) was 70% of the pre-exercise $Q_{tw,pot}$ and did not differ between conditions (mean ERT 75 ± 25 N; $P = 0.811$). Post-exercise ERT was reduced in AH (52 ± 27 N, $P = 0.049$), but was unchanged in N and CH ($P \geq 0.107$).

Corticomotor responses. rMT in AH, N and CH was 54 ± 5, 53 ± 3 and 51 ± 6% maximum stimulator output ($P = 0.276$) respectively. During CH, resting MEP amplitude was twofold greater compared to AH ($P = 0.014$) and N ($P = 0.014$). Exercise elicited a reduction in resting MEP amplitude in CH ($P = 0.022$), but not AH ($P = 0.346$) or N ($P = 0.369$). MEPs evoked during brief knee extensor contractions at 100, 75 and 50% MVC pre-exercise were higher in CH compared to AH ($P < 0.020$) and N ($P < 0.030$) (see also Fig. 4). MEPs evoked during the brief knee extensor contractions (50–100% MVC) post-exercise were not significantly different from pre-exercise values in any condition. MEP amplitude, however, was higher post-exercise during CH compared to AH (50% MVC, $P = 0.018$; 75% MVC, $P = 0.030$) and N (50% MVC, $P = 0.034$). The MEP/M_{max} ratio increased for within contraction responses during CH (vs. AH 50 and 75% MVC; $P \leq 0.014$ and N 50% MVC; $P = 0.019$) (Table 2). The CSP did not differ between conditions pre-exercise (pooled average, 186 ± 47 ms; $P = 0.880$) or post-exercise (pooled average, 185 ± 50 ms; $P = 0.760$). Baseline cortical voluntary activation did not differ between conditions (AH, 93 ± 5%; N, 97 ± 3%; CH, 93 ± 6%; $P = 0.310$) (Fig. 5). Cortical voluntary activation was reduced post-exercise in AH (Δ11%, $P = 0.014$), but not in N (Δ4%, $P = 0.298$) or CH

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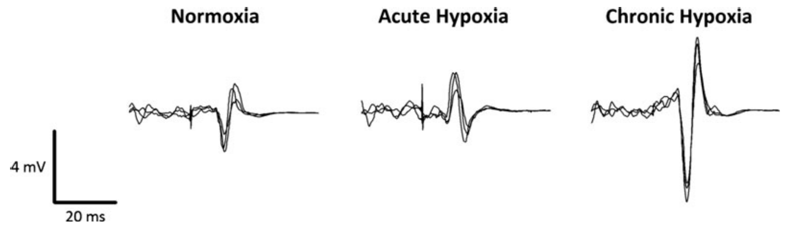

Figure 4 Representative MEPs evoked during knee extensor contractions at 50% MVC before exercise in each condition. Traces are shown from a representative participant in each condition; 8 stimuli were delivered from which an average value was obtained. Note the increase in MEP amplitude (corticospinal excitability) after acclimatization.

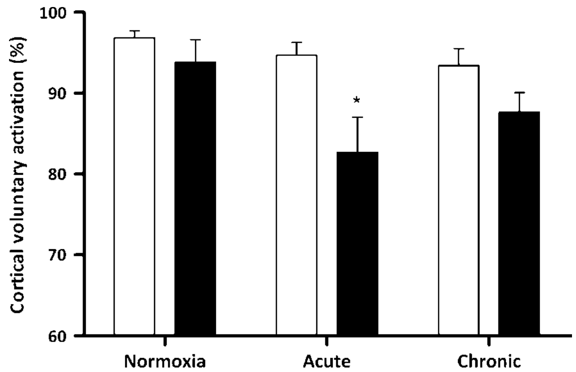

Figure 5 Cortical voluntary activation measured before (open bars) and immediately after (<2.5 min; closed bars) constant-load exercise (131 W) in normoxia (N), acute hypoxia (AH) and chronic hypoxia (CH). **P* < 0.05 pre- versus post-exercise.

(Δ6%, $P = 0.174$); the decrease in AH was greater compared to N ($P = 0.022$) (Fig. 5).

Discussion

The aim of the present study was to assess corticospinal excitability and supraspinal fatigue after locomotor exercise in chronic hypoxia. The main finding was that exercise-induced supraspinal fatigue, as quantified via changes in cortical voluntary activation, was attenuated after 2 weeks of acclimatization to high altitude whereas it was exacerbated in AH versus N. Importantly, the diminished level of central fatigue in CH occurred in parallel with improvements in cerebral haemodynamics and arterial oxygenation (increased C_aO_2 and S_pO_2) brought about by the 2 weeks at altitude. Moreover, the attenuated development of central fatigue occurred in line with a substantial increase in corticospinal excitability. This latter finding suggests that a period of acclimatization modifies the integrity of the corticospinal tract. We confirm our hypothesis that acclimatization to altitude reduces the level of exercise-induced central fatigue and that this is attributable, at least in part, to an increased overall excitability of the brain to muscle pathway.

Supraspinal fatigue

A key aim of the present study was to determine the effect of acclimatization on the development of central fatigue assessed after exercise. We hypothesized that improvements in cerebral oxygenation known to occur after a prolonged stay at altitude would bring about positive modifications on the development of central fatigue. We show that the development of supraspinal fatigue during locomotor exercise is recovered after 2 weeks at high altitude and similar to that observed in normoxia. Thus, the adaptive processes that take place during acclimatization to high altitude seemingly protect healthy humans against the development of supraspinal fatigue.

Corticomotor responses

The present study found no change in corticospinal excitability (Δ resting MEP) in AH, a finding which is in line with literature utilizing varying severities of hypoxia ($F_IO_2 = 0.14$–0.10; resting $S_pO_2 = 93$–74%) for as little as 10 min to 1 h (Goodall *et al.* 2010, Millet *et al.* 2012, Rupp *et al.* 2012). However, Szubski *et al.* (2006) reported increased corticospinal excitability, expressed as a reduced rMT (not ΔMEP), after approximately 30 min of breathing hypoxic air ($F_IO_2 = 0.12$; resting $S_pO_2 = 75\%$). Moreover, the present study found a twofold increase in corticospinal excitability after 14 days acclimatization to severe altitude (5260 m, equivalent to F_IO_2 0.105; resting $S_pO_2 = 91 \pm 2\%$) with accompanying increases in the MEP/M_{max} ratio, suggesting that the increases in MEP size were due to adaptive mechanisms within spinal and/or supraspinal sites. Similarly, Rupp *et al.* (2012) found a 26% increase in corticospinal excitability (ΔMEP amplitude) after 3 h of exposure to normobaric hypoxia ($F_IO_2 = 0.12$; resting $S_pO_2 = 86\%$), demonstrating a time-dependent, hypoxia-induced modification in the brain to muscle pathway. Thus, a prolonged stay at altitude modifies the integrity of the corticospinal pathway which may contribute to reduce the level of central fatigue; however, a

duration-dependent adaptation cannot yet be established with certainty.

TMS over the motor cortex preferentially activates corticospinal neurones trans-synaptically through excitatory interneurones and corticocortical axons (Di Lazzaro *et al.* 1998). The response to TMS critically depends on membrane excitability of motor cortical neurones and ion-channel function (Rothwell *et al.* 1991, Boroojerdi *et al.* 2001). *In vitro* investigations using isolated cerebral neurones from rats demonstrate that ion-channel function is affected by O_2 availability and that neuronal hyperexcitability is the consequence of chronic hypoxia (Donnelly *et al.* 1992). A heightened neural response is necessary to maintain membrane integrity and ionic homoeostasis that occur from a period of insufficient metabolic activity (Nieber *et al.* 1999). Thus, the twofold increase in MEP observed in the present study might be due to facilitated cortical neurones acting to restore the loss of neuronal activity associated with a prolonged exposure to altitude. Additionally, an increased level of muscle sympathetic nerve activity (peroneal microneurography) has been reported during a prolonged stay at the same altitude as in the present study (Hansen & Sander 2003). That study showed a significant increase in muscle sympathetic nerve activity just 3 days after exposure to high altitude, suggesting that the prolonged stay induced a striking and long-lasting sympathetic over-activity. More recently, Buharin *et al.* (2013) found that a transient increase in sympathetic nerve activity (induced via lower body negative pressure) enhances corticospinal excitability as identified using TMS. The mechanism responsible for the increase in corticospinal excitability was postulated to be due to an increased concentration of noradrenaline, a monoamine that is known to increase exponentially during sustained periods at altitudes exceeding 4000 m (Cunningham *et al.* 1965, Mazzeo *et al.* 1994). Thus, the increased corticospinal excitability observed following 2 weeks of acclimatization in the present study might be attributable, at least in part, to a heightened sympathetic nerve activity and associated increases in corticospinal excitability as well as hyperexcitable cerebral neurones. The increased corticospinal excitability in this investigation occurred in line with no symptoms of mountain sickness, a finding that opposes that of Miscio *et al.* (2009). Miscio *et al.* (2009) found that exposure to high altitude changes cortical excitability by affecting both inhibitory and excitatory circuits and that this is reflected in acute mountain sickness symptoms. This conclusion was based on a group of participants who resided at 4554 m for only 3–5 days, a time frame in which acute mountain sickness is said to be most prominent (Hackett & Roach 2001) and much shorter than the present study.

Despite substantial differences in end-exercise peripheral fatigue, CSP duration immediately after exercise (i.e. pre-to post-exercise change) was similar in all conditions. This suggests that locomotor exercise in N, AH and CH does not influence intracortical inhibition. These findings are in agreement with investigations using locomotor exercise in N and AH (Sidhu *et al.* 2009b, Goodall *et al.* 2012). However, Oliviero *et al.* (2002) reported decreased intracortical inhibition and CSP duration in chronic hypoxaemic patients with COPD. These changes, mediated by cerebral GABA receptors, were reversed after 3–4 months of O_2 therapy, demonstrating that the changes were O_2 sensitive. However, factors other than chronic hypoxaemia might influence intracortical inhibition in patients with COPD, making it difficult to quantify the influence that chronic hypoxaemia has on cortical inhibition.

On balance, we judge the increased corticospinal excitability in CH noted in the present study to be the result of adaptations in ion-channel function and increases in circulating catecholamines serving to facilitate neurotransmission rather than mechanisms related to intracortical inhibition (Palange 1998, Nieber *et al.* 1999, Buharin *et al.* 2013).

Haematological and cerebrovascular responses

Upon initial exposure to high altitude, acute hypoxia dilates cerebral arterioles, thereby overriding the vasoconstrictive effect of hyperventilation-associated hypocapnia (Iwasaki *et al.* 2011). During a prolonged stay at altitude, hypocapnia further develops and arterial hypoxaemia is ameliorated, as reflected by increases in arterial [Hb], PO_2 and O_2 saturation (Fig. 3). Furthermore, the increase in P_aO_2 and further decrease in P_aCO_2 with acclimatization causes relative vasoconstriction, reducing CBF down to SL values (Subudhi *et al.* 2013). We estimated an index of cerebral O_2 delivery using the product of MCAV and C_aO_2. Our data demonstrate a reduced cerebral O_2 delivery index during exercise in AH compared to N; however, an improved cerebral O_2 delivery index was evident after 2 weeks of acclimatization (Fig. 3). The data in AH support a relationship between cerebral O_2 delivery and supraspinal fatigue (Goodall *et al.* 2012). The calculation of C_aO_2 during exercise from resting [Hb] should be interpreted with caution as a haemoconcentration could have impacted this measure. At sea level, the haemoconcentration accompanying maximal exercise for approximately 10 min is counterbalanced by the concomitant exercise-induced arterial hypoxaemia with the net effect of similar C_aO_2 at rest and during exercise (Amann *et al.* 2006a). At altitude, despite significant haemoconcentration, C_aO_2 actually falls from

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rest to submaximal/maximal exercise by 10–25% (Calbet *et al.* 2003). This would suggest that exercise C_aO_2 calculations, based on a resting C_aO_2 measure, might actually overestimate C_aO_2 measured during exercise at altitude. Furthermore, we assumed that MCA diameter would remain constant in hypoxia (Poulin & Robbins 1996, Serrador *et al.* 2000). While there is evidence of MCA dilatation at rest in hypoxia (Wilson *et al.* 2011, Willie *et al.* 2012), there is currently no evidence of MCA dilatation during intense exercise accompanied with substantial exercise-induced hyperventilation and associated hypocapnia. We acknowledge, however, that our measurements of blood velocity (rather than flow) must be interpreted with caution.

We found acclimatization-induced increases in O_2 saturation and content (Fig. 3). Furthermore, arterial O_2 tension increased from AH to CH (approx. 39–59 mmHg). Subudhi *et al.* (2013) has shown resting cerebral O_2 delivery to be maintained at levels observed in N during AH and CH, although it is presumed that the delivery of O_2 to the mitochondria within the parenchyma will be reduced because the driving gradient for diffusion from capillary to tissue is the PO_2 difference between capillary and tissue (Xu & Lamanna 2006). The tissue PO_2 would be close to zero; thus, the driving force is essentially the P_aO_2. In the present study, the P_aO_2 increased in line with acclimatization, thereby improving the gradient for diffusion and perhaps restoring brain tissue O_2 tension to pre-hypoxic levels (Dunn *et al.* 2000). Thus, we postulate that the lack of central fatigue in chronic hypoxia may be related to increases in brain tissue O_2 tension. However, the link between increases in P_aO_2 and C_aO_2 and the reduction in central fatigue that occurs after a period of acclimatization warrants further investigation.

Technical considerations

Exercising in a hypobaric environment was not feasible for the trials in AH. Thus, the two modes of hypoxia (normobaric [AH] vs. hypobaric [CH]) differed. The literature concerning the responses in normobaric and hypobaric hypoxia is equivocal and readers are directed elsewhere to a point:counterpoint debate (Girard *et al.* 2012). Briefly, it was proposed that evidence is growing, suggesting that hypobaric hypoxia affects responses (ventilation, fluid balance, acute mountain sickness and performance) to a greater extent than normobaric hypoxia (Girard *et al.* 2012). However, this argument was opposed by the fact that in terms of O_2 sensing, hypobaric hypoxia does not induce different responses compared to normobaric hypoxia (Mounier & Brugniaux 2012). Moreover, it is unknown how any such differences which might exist between hypobaric and normobaric hypoxia may affect indices of exercise-induced fatigue. We set the F_IO_2 (0.105) at sea level to obtain the same P_IO_2 (approx. 74 mmHg) that was expected at the subsequent altitude in Bolivia (5260 m).

In line with other investigations that have measured exercise-induced fatigue of the knee extensors (Sidhu *et al.* 2009b, Goodall *et al.* 2010, 2012, Rossman *et al.* 2012), measurements were made within 2.5 min after exercise termination. Corticospinal excitability associated with maximal single muscle contractions recovers within 1 min post-exercise (Taylor *et al.* 1999). Thus, the present experimental design, utilizing whole-body exercise, might not have captured all elements of central fatigue. However, the methods and time to assess fatigue after exercise in all three conditions were identical, and even though our measurements were made more than 1 min post-exercise, significant differences were observed, testifying to the strength of our data.

Conclusion

The novel finding was that supraspinal fatigue, present after exercise in acute hypoxia, was attenuated after a period of acclimatization to high altitude. Importantly, the reduced development of central fatigue in chronic hypoxia occurred in parallel with an increase in the excitability of the brain to muscle pathway consequent to an increased cerebral O_2 delivery. The attenuated rate of development of central fatigue in chronic hypoxia might explain, at least in part, the improvements in locomotor exercise performance that are commonly observed after acclimatization to high altitude.

Author contributions

SG, RT and MA contributed to conception and design of the experiments, data collection, data analysis, data interpretation, manuscript drafting and editorial process. ER contributed to conception and design of the experiments, data interpretation and manuscript revision. AL contributed to data collection. LR contributed to conception and design of the experiments, data interpretation, manuscript drafting and revision. AL, AS and RR conceived, designed and executed the AltitudeOmics study of which the present study was a part, and contributed to manuscript revision. AS also contributed to data collection and data interpretation. All authors approved the final version of the manuscript.

Conflict of interest

Nothing to declare.

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AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans

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Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC. AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans. *J Appl Physiol* 115: 634–642, 2013. First published June 27, 2013; doi:10.1152/japplphysiol.00606.2013.—The development of muscle fatigue is oxygen (O_2)-delivery sensitive [arterial O_2 content (C_aO_2) × limb blood flow (Q_L)]. Locomotor exercise in acute hypoxia (AH) is, compared with sea level (SL), associated with reduced C_aO_2 and exaggerated inspiratory muscle work (W_{insp}), which impairs Q_L, both of which exacerbate fatigue individually by compromising O_2 delivery. Since chronic hypoxia (CH) normalizes C_aO_2 but exacerbates W_{insp}, we investigated the consequences of a 14-day exposure to high altitude on exercise-induced locomotor muscle fatigue. Eight subjects performed the identical constant-load cycling exercise (138 ± 14 W; 11 ± 1 min) at SL (partial pressure of inspired O_2, 147.1 ± 0.5 Torr), in AH (73.8 ± 0.2 Torr), and in CH (75.7 ± 0.1 Torr). Peripheral fatigue was expressed as pre- to postexercise percent reduction in electrically evoked potentiated quadriceps twitch force ($\Delta Q_{tw,pot}$). Central fatigue was expressed as the exercise-induced percent decrease in voluntary muscle activation (ΔVA). Resting C_aO_2 at SL and CH was similar, but C_aO_2 in AH was lower compared with SL and CH (17.3 ± 0.5, 19.3 ± 0.7, 20.3 ± 1.3 ml O_2/dl, respectively). W_{insp} during exercise increased with acclimatization (SL: 387 ± 36, AH: 503 ± 53, CH: 608 ± 67 $cmH_2O \cdot s^{-1} \cdot min^{-1}$; $P < 0.01$). Exercise at SL did not induce central or peripheral fatigue. $\Delta Q_{tw,pot}$ was significant but similar in AH and CH (21 ± 2% and 19 ± 3%; $P = 0.24$). ΔVA was significant in both hypoxic conditions but smaller in CH vs. AH (4 ± 1% vs. 8 ± 2%; $P < 0.05$). In conclusion, acclimatization to severe altitude does not attenuate the substantial impact of hypoxia on the development of peripheral fatigue. In contrast, acclimatization attenuates, but does not eliminate, the exacerbation of central fatigue associated with exercise in severe AH.

altitude; respiratory muscle work; arterial O_2 content; cerebral blood flow

THE DEVELOPMENT OF LOCOMOTOR muscle fatigue during whole-body endurance exercise is highly sensitive to the delivery of oxygen [O_2; arterial O_2 content (C_aO_2) × leg blood flow (Q_L)]. Specifically, blunted O_2 delivery exaggerates, and augmented O_2 delivery attenuates the rate of development of locomotor muscle fatigue during exercise (1).

Acute exposure to hypoxia (AH) has a substantial impact on the two determinants of leg muscle O_2 delivery during strenuous locomotor exercise. First, despite a marked hyperventilatory response, arterial partial pressure of O_2 [PO_2 (P_aO_2)] and arterial hemoglobin saturation (S_aO_2) fall below sea level (SL) values and cause a significant reduction in C_aO_2. In addition, inspiratory muscle work (W_{insp}) is increased substantially at any given workload in hypoxia (2, 58), and these high levels of W_{insp} compromise, in a dose-dependent manner, Q_L during exercise (34). Each of these two determinants of leg muscle O_2 delivery, namely C_aO_2 and Q_L, accounts for, substantially and independently, the accelerated development of locomotor muscle fatigue in hypoxia (2).

During prolonged exposure to altitude, a progressive, time-dependent hyperventilation, which increases alveolar PO_2, occurs over the initial hours and days and advances more gradually over the ensuing 1–2 wk of acclimatization (56). This ventilatory acclimatization adds to an accompanying reduction in the alveolar-arterial O_2 gradient, which combined, substantially improves arterial oxygenation during exercise by increasing P_aO_2 and S_aO_2 (9, 13). Furthermore, chronic exposure to hypoxia (CH) is accompanied by erythropoiesis, and the combination of an increased hemoglobin concentration ([Hb]) plus improved oxygenation may serve to restore resting SL C_aO_2 (8, 13). In contrast to this beneficial effect on O_2 delivery, Q_L, during intense leg exercise at a given submaximal absolute workload, has been suggested to decline from SL to CH (8, 49, 64). The net effect of these acclimatization-induced, opposing consequences on leg O_2 delivery depends on the degree to which the increase in C_aO_2 can counterbalance potential reductions in Q_L. It has been documented previously that at a given absolute workload, locomotor muscle O_2 delivery is reduced from SL to AH with no further changes following acclimatization (Pikes Peak, 4,300 m) (8, 64). Therefore, given the critical role of muscle O_2 delivery in the development of fatigue, it could be argued that peripheral fatigue during constant-load endurance exercise is exacerbated in AH (vs. SL) and does not improve further during prolonged acclimatization. On the other hand, studies conducted at the same location as the present experiments [Mt. Chacaltaya (Bolivia), 5,260 m] document a reduction in locomotor muscle O_2 delivery from SL to AH and a full recovery following prolonged exposure, with the net effect of similar values in SL and CH (13). Based on these findings, it could be argued that the development of peripheral fatigue during constant-load endurance exercise is fastened in AH but recovers to SL values in CH.

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In this study, we sought to quantify exercise-induced locomotor muscle fatigue induced by the identical constant-load cycling trial performed at SL, in AH, and in CH (following 14 days at 5,260 m) to clarify the effects of acclimatization. We hypothesized that fatigue is, compared with SL, exacerbated significantly in AH and that altitude acclimatization would alleviate this impact.

METHODS

This study was conducted as part of the AltitudeOmics project, examining the integrative physiology of human responses to hypoxia. All procedures conformed to the Declaration of Helsinki and were approved by the Universities of Colorado, Oregon, and Utah Institutional Review Boards and the U.S. Department of Defense Human Research Protection Program Office. All subjects were born and raised below 1,500 m and had not traveled to elevations >1,000 m for 3 mo before the experiments. Eight subjects (age 21 ± 1 y, body weight 69 ± 11 kg, height 176 ± 10 cm) were studied at SL and following 14 days of altitude acclimatization at 5,260 m on Mt. Chacaltaya. At high altitude, subjects did not follow a systematic exercise-training program but were given the opportunity to participate, on a voluntary basis, in light hikes around the campsite (no significant change in altitude).

Experimental Protocol

All participants were familiarized thoroughly with various experimental procedures involved in this investigation. The SL experiments of the present study were conducted ~130 m above SL [Eugene, OR; barometric pressure (BP) 750.0 ± 2.2 Torr]. The experiments in AH were conducted at the same altitude, while breathing a gas mixture containing 10.5% O_2 balance nitrogen, and experiments in CH were conducted on the 14th day of acclimatization at 5,260 m (BP 408.9 ± 0.7 Torr). Two participants were tested every morning. To assure that all subjects were tested exactly on *day 14* after arrival on the mountain, the groups' transport to the mountain was staged, i.e., two new participants arrived every day. SL peak power output (W_{peak}) was obtained from a maximal incremental exercise test (70, 100, 130, and 160 W for 3 min, each followed by 15 W/min increases thereafter) on a computer-controlled bicycle ergometer (Velotron, Dynafit; RacerMate, Seattle, WA). The experimental trial consisted of the identical constant-load cycling exercise (same absolute workload and duration) in each condition. Preliminary experiments (using different subjects), conducted to identify a workload that causes voluntary exhaustion between 8 and 12 min when acutely exposed to 5,260 m, revealed that a constant workload equal to 50% of SL W_{peak} was required to reach this goal. Based on this, the workload during the experimental trials was set to equal 50% (138 ± 14 W) of the subjects' SL W_{peak} (275 ± 14 W). Since an individual's endurance/aerobic capacity is lowest in AH (vs. SL and CH) (13), the first trial was performed to voluntary exhaustion in AH, and the achieved time (10.6 ± 0.7 min) was then used for all subsequent trials. A 5-min warm-up at 10% W_{peak} (27 ± 8 W) preceded each trial. Throughout exercise, subjects were instructed to maintain their preferred pedal frequency, as determined during the practice sessions (88 ± 3 rpm). Neuromuscular function was assessed before and within 2.5 min after exercise. During these procedures, subjects breathed ambient air at SL and in CH and a gas mixture (10.5% O_2) in AH.

Exercise Responses

Pulmonary ventilation (V_E) and gas exchange were measured at rest and throughout exercise using an open circuit system (Ultima PFX; Medical Graphics, St. Paul, MN, and O2cap; Oxigraf, Mountain View, CA). Arterial O_2 saturation (S_pO_2) was estimated continuously at rest and during exercise using a pulse oximeter (Nellcor N-200; Pleasanton, CA) with adhesive forehead sensors. A correction factor based on arterial blood gases was used to adjust for the nonlinearity associated with the obtained pulse oximeter values (error between 60% and 80% saturation: 6%; error between >90% saturation: 3%). Heart rate was measured from the R–R interval of an ECG, using a three-lead arrangement. Ratings of perceived exertion were obtained using Borg's modified CR10 scale (10). [Hb] was measured (Radiometer OSM-3) in resting arterial blood samples collected at SL and on the 16th day at 5,260 m. C_aO_2 was estimated as 1.39 [Hb] × (S_pO_2/100). During all constant workload trials, esophageal pressure (P_{es}) was measured via a nasopharyngeal balloon (Cooper Surgical, Trumbull, CT), using standard procedures (7). To estimate W_{insp}, P_{es} was integrated over the period of inspiratory flow, and the results were multiplied by respiratory frequency (f_R) and labeled the inspiratory muscle pressure-time product. Vastus lateralis oxygenation was assessed using a multichannel near-infrared spectroscopy (NIRS) instrument (Oxymon Mk III; Artinis, Zetten, The Netherlands). As described previously (5), a NIR emitter and detector pair was affixed over the belly of the left vastus lateralis muscle (~15 cm proximal and 5 cm lateral to the midline of the superior border of the patella), using a spacer with an optode distance of 5.0 cm. Probes were secured to the skin using double-sided tape and shielded from light using elastic bandages. The Beer-Lambert Law was used to calculate micrometer changes in tissue oxygenation [oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb)] across time. using received optical densities from two continuous wavelengths of NIR light (780 and 850 nm) and a fixed differential path-length factor of 4.95 (26). Total hemoglobin (THb) was calculated as the sum of [O_2Hb] and [HHb] changes to give an index of change in regional blood volume (59). Data were recorded continuously at 10 Hz and expressed relative to the resting baseline recorded in each experimental condition. Mean cerebral blood flow (CBF) was estimated from blood velocity (CBFv) in the left middle cerebral artery (MCA; 50 ± 4 mm deep), determined using a 2-MHz transcranial Doppler (Spencer Technologies, Seattle, WA). An index of cerebral O_2 delivery was calculated as the product of CBFv and C_aO_2. Changes in CBFv were assumed to reflect changes in CBF, based on evidence that the MCA changes minimally in response to hypoxia and hypocapnia (47, 54). The validity of this assumption at altitude has been challenged recently (62). Evidence of MCA dilation was demonstrated in subjects at altitudes above 6,400 m, but no changes in MCA diameter were observed at altitudes comparable with the present study (<5,300 m) (63). We acknowledge that these measurements must be interpreted with caution until definitive studies of MCA diameter at altitude are conducted.

Expiratory Flow Limitations and Lung Volume Responses

Expiratory flow limitations. Subjects performed three maximal volitional flow-volume (FV) maneuvers before and after exercise (after assessment of neuromuscular function). Exercise tidal FV loops (FVLs) were plotted within the best of the six maximal loops (MFVLs), based on measured inspiratory capacity (IC) maneuvers (rest, 3 min of exercise, and immediately before the termination of exercise). Acceptable IC maneuvers during exercise required that peak inspiratory P_{es} match that obtained at rest. The amount of expiratory flow limitation was defined as the percentage of the tidal volume (V_T) that met the boundary of the expiratory portion of the MFVL (38).

Lung volumes. Functional residual capacity (FRC) was measured in a body plethysmograph (Platinum Elite Series; Medical Graphics), and total lung capacity (TLC) was calculated as the sum of FRC and IC. End-expiratory lung volume (EELV) was determined by subtracting the maximal IC, as measured during exercise from TLC, as measured at rest. End-inspiratory lung volume (EILV) was calculated as the sum of EELV and V_T. Inspiratory reserve volume, during exercise, was calculated by subtracting EILV from TLC, and expiratory reserve volume, during exercise, was determined by subtracting the residual volume from EELV.

Force and Compound Muscle Action Potentials

Knee-extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Tedea, Basingstoke, UK). The load cell was fixed to a custom-built chair and connected to a noncompliant cuff, attached around the participant's right leg, just superior to the ankle malleoli. Participants sat upright in the chair with the hips and knees at 90° of flexion. Compound muscle action potentials (M-waves) were recorded from surface electrodes placed 2 cm apart over the vastus lateralis muscle belly. A reference electrode was placed over the patella. Evoked signals were amplified [gain: 1,000; force: custom-built bridge amplifier; electromyographic (EMG): PowerLab 26T; ADInstruments (Oxfordshire, UK)], band-pass filtered (EMG only: 20–2,000 Hz), digitized (4 kHz; PowerLab 26T, ADInstruments), acquired, and later analyzed (LabChart v7.0; ADInstruments) for peak-to-peak amplitude.

Neuromuscular Function

Force and EMG variables were assessed before and immediately (<2.5 min) after each trial. Before each trial, maximum voluntary contraction (MVC) force was determined from three control contractions. Femoral nerve stimulation was delivered during each 5-s MVC, and an additional stimulus was delivered after the MVC to determine the potentiated quadriceps twitch force ($Q_{tw,pot}$) and voluntary muscle activation (VA) (42). Briefly, the force produced during the superimposed twitch (SIT), delivered within 0.5 s of attaining peak force during the MVC, was to be compared with the force produced by the single twitch, delivered during relaxation, ~2 s after the MVC: VA (%) = [1 − (SIT/$Q_{tw,pot}$)] × 100. The contraction sets were repeated three times, with 30 s between each set. Visual feedback of the target force was provided via a computer monitor.

Femoral nerve stimulation. Single electrical stimuli (200 μs pulse width) were delivered to the right femoral nerve via surface electrodes (32 mm diameter; CF3200; Nidd Valley Medical, North Yorkshire, UK) and a constant-current stimulator (DS7AH; Digitimer, Welwyn Garden City, Hertfordshire, UK). The cathode was positioned over the nerve, high in the femoral triangle; the anode was placed midway between the greater trochanter and the iliac crest (32). The site of stimulation that produced the largest resting twitch amplitude and M-wave was located. Single stimuli were delivered, beginning at 100 mA and increasing by 20 mA, until plateaus occurred in twitch amplitude and M-wave. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current, 250 ± 55 mA). Muscle contractility was assessed for each potentiated twitch as twitch amplitude ($Q_{tw,pot}$: peak force − onset force), maximum rate of force development (MRFD), contraction time, maximum relaxation rate (MRR), and one-half relaxation time ($RT_{0.5}$). Sarcolemmal membrane excitability was inferred from the peak-to-peak amplitude of the electrically evoked M-wave (27).

Reliability Measures

On a separate day, measures of neuromuscular function were repeated twice in all subjects at SL. The two assessment procedures were separated by a 2-min walk around the laboratory, followed by a 5-min rest period. Coefficient of variation (CV) and Pearson product-moment correlation coefficients (*r*) were calculated to evaluate test-retest error (precision) and test-retest reliability of the neuromuscular function-assessment procedure. All correlations were significant and indicated; in combination with the CVs, acceptable degrees of reproducibility include: MVC, CV = 3.1%, $r = 0.97$; $Q_{tw,pot}$, CV = 4.1%, $r = 0.98$; M-wave peak, CV = 4.8%, $r = 0.98$; VA, CV = 3.3%, $r = 0.77$.

Statistical Analysis

A one-way repeated-measures ANOVA was performed to evaluate differences among trials. A least-significance difference test identified the means that were significantly different with $P < 0.05$. Results are expressed as mean ± SE.

RESULTS

C_aO_2 and Cerebral O_2 Delivery

C_aO_2 at rest was significantly lower in AH compared with SL and CH (17.3 ± 0.5, 19.3 ± 0.7, 20.3 ± 1.3 ml O_2/dl, respectively). Acclimatization to altitude significantly increased [Hb] and S_pO_2, resulting in similar C_aO_2 at SL and in CH ($P = 0.16$). Resting CBFv was similar among SL, AH, and CH (50.5 ± 3.7, 52.7 ± 2.3, and 55.7 ± 3.0 cm/s, respectively; $P = 0.45$). In all three conditions, CBFv increased significantly from rest to the final minute of exercise (22 ± 3%, 39 ± 6%, and 28 ± 5% for SL, AH, and CH, respectively; Table 1). The percent increase was significantly greater in AH compared with that observed at SL and in CH. The cerebral O_2 delivery index during the last minute of exercise was 18 ± 5% lower in AH vs. SL (Table 1) and 17 ± 8% greater in CH vs. SL (Table 1).

Ventilatory Effects

Ventilatory response. AH increased W_{insp} work by 34 ± 8% above that at SL ($P < 0.01$) and dropped S_pO_2 by 36 ± 3% during the final minute of exercise. Following 14 days of acclimatization, W_{insp} was increased further by 23 ± 8% from AH, and S_pO_2, during the final minute of exercise, was 36 ± 5% higher in CH vs. AH. Breathing frequency and V_E rose

Table 1. *Mean responses to the final minute of exercise (138 ± 14 W, 10.6 ± 0.7 min)*

	Sea Level	Acute Hypoxia	Chronic Hypoxia
HR, beats/min	152 ± 5	174 ± 4*	166 ± 4*†
V_E, l min^{-1}	64 ± 4	113 ± 8*	133 ± 10*†
f_R, breaths min^{-1}	32 ± 2	50 ± 3*	54 ± 3*
V_T, liter	2.0 ± 0.1	2.2 ± 0.2	2.6 ± 0.2*†
$\dot{V}O_2$, l min^{-1}	2.58 ± 0.19	2.44 ± 0.19*	2.39 ± 0.16*†
$\dot{V}CO_2$, l min^{-1}	2.51 ± 0.22	2.81 ± 0.21*	2.40 ± 0.15*†
$V_E/\dot{V}O_2$	25 ± 1	50 ± 4*	56 ± 3*†
$V_E/\dot{V}CO_2$	26 ± 1	41 ± 2*	58 ± 3*†
S_pO_2, %	94.1 ± 1.0	62.2 ± 1.8*	75.6 ± 1.2*†
CBFv, cm/s	59.1 ± 4.8	74.2 ± 3.8*	73.2 ± 3.4*
Cerebral O_2 delivery, a.u.	1,105 ± 62	895 ± 40*	1,289 ± 42*†
T_i/T_{tot}	0.35 ± 0.01	0.39 ± 0.01*	0.39 ± 0.01*
T_e, s	1.30 ± 0.08	0.74 ± 0.05*	0.70 ± 0.04*
W_{insp}, cmH$_2$O · s^{-1} · min^{-1}	387 ± 36	503 ± 53*	608 ± 67*†
IC, liter	3.29 ± 0.22	3.13 ± 0.23	3.60 ± 0.23*
V_T/IC	0.60 ± 0.03	0.68 ± 0.02*	0.72 ± 0.02*†
IRV, liter	1.30 ± 0.14	0.99 ± 0.13*	0.96 ± 0.05*
ERV, liter	1.98 ± 0.25	2.14 ± 0.29	1.67 ± 0.25*†
EILV, %TLC	80.5 ± 1.6	85.4 ± 1.7*	85.2 ± 0.9*
EELV, %TLC	51.5 ± 1.8	53.8 ± 2.5	46.9 ± 2.1*†
Expiratory flow limitation, *n* out of 8 subjects	0/8	2/8	4/8
RPE	12.3 ± 1.0	19.8 ± 0.1*	17.9 ± 0.6*†
Dyspnea	11.5 ± 0.7	19.5 ± 0.2*	19.3 ± 0.2*

HR, heart rate; V_E, minute ventilation; f_R, breathing frequency; V_T, tidal volume; $\dot{V}O_2$, maximum oxygen (O_2) uptake; $\dot{V}CO_2$, carbon dioxide production; S_pO_2, arterial O_2 saturation; CBFv, cerebral blood flow velocity; T_i, duration of inspiration; T_{tot}, duration of entire breath; T_e, duration of expiration; W_{insp}, inspiratory muscle work; IC, inspiratory capacity; IRV, inspiratory reserve volume; ERV, expiratory reserve volume; EILV, end-inspiratory lung volume; TLC, total lung capacity; EELV, end-expiratory lung volume; RPE, rating of perceived exertion. *$P < 0.05$ vs. sea level; †$P < 0.05$ vs. acute hypoxia, $n = 8$.

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substantially over the time of exercise in AH and CH, and V_E was, during the final minute, 79 ± 13% and 110 ± 12%, respectively, higher compared with SL ($P < 0.01$). Pulmonary V_E during the final minute of exercise was 19 ± 4% higher in CH vs. AH ($P < 0.01$). Compared with SL, O_2 uptake, during the final minute of exercise, was 5 ± 2% and 7 ± 2% lower in AH and CH, respectively (both $P < 0.05$; Fig. 1).

Expiratory flow limitation. At SL, exercise flow rates during tidal breathing were well within the MFVL in all eight subjects. At end-exercise in AH, 6–51% of the V_T in two of the eight subjects reached flow limitation, as lung volume approached end-expiration. As V_E increased further in CH, expiratory flow rate became more limited, and 10–64% of the V_T in four of the eight subjects met the limit imposed by the MFVL.

Membrane Excitability and Contractile Function

M-waves. As a measure of membrane excitability we examined pre- vs. postexercise vastus lateralis M-wave amplitudes in conjunction with the quadriceps muscle mechanical properties. Pre-exercise M-wave amplitudes were similar in all three conditions (10.2 ± 1.0 mV, 9.4 ± 0.7 mV, and 12.9 ± 1.8 mV for SL, AH, and CH, respectively; $P = 0.15$). Postexercise M-wave amplitudes were unchanged from pre-exercise baseline values at SL and in AH (10.2 ± 1.0 mV and 9.6 ± 0.9 mV, respectively; $P > 0.3$). However, following exercise in CH, M-wave amplitudes (7.8 ± 2.1 mV) were reduced significantly from pre-exercise baseline levels (range: 1–18%; $P < 0.01$).

Quadriceps twitch force. Pre-exercise $Q_{tw,pot}$ was similar in all three conditions (106 ± 4 N, 109 ± 4 N, and 110 ± 5 N for SL, AH, and CH, respectively; $P = 0.18$). Exercise in both hypoxic conditions caused a substantial ($P < 0.01$) but similar ($P = 0.14$) reduction in $Q_{tw,pot}$ in all eight subjects. In contrast, exercise at SL did not induce measurable locomotor muscle fatigue; the postexercise $Q_{tw,pot}$ was similar to pre-exercise baseline.

MVC force. Pre-exercise MVC was similar in all three conditions (391 ± 30 N, 394 ± 25 N, and 372 ± 30 N for SL, AH, and CH, respectively; $P = 0.21$). At SL, postexercise MVC was similar to pre-exercise baseline ($P = 0.42$). In contrast, exercise in AH and CH caused a substantial reduction in MVC in all eight subjects. However, the exercise-induced reduction in MVC was 30 ± 9% less in CH vs. AH ($P < 0.05$).

Muscle activation. Pre-exercise baseline values were similar in all three conditions (94 ± 1%, 94 ± 1%, and 93 ± 1% for SL, AH, and CH, respectively; $P = 0.19$). Following the exercise at SL, muscle activation was unchanged from pre-exercise baseline ($P = 0.88$). In both AH and CH, postexercise muscle activation was significantly lower compared with pre-exercise baseline values. However, the pre- to postexercise decrease in muscle activation was 52 ± 12% less in CH vs. AH ($P < 0.01$).

Within-twitch measurements. MRFD, MRR, and $RT_{0.5}$ complement the findings reported for $Q_{tw,pot}$. The pre- to postexercise changes in within-twitch measurements of MRFD, MRR, and $RT_{0.5}$ were similar in CH vs. AH.

Vastus Lateralis Tissue Oxygenation

O_2Hb was unchanged from baseline to warm-up at SL ($P = 0.40$) but decreased in AH ($P < 0.05$) and CH ($P = 0.05$). Compared with baseline, O_2Hb was unchanged during the final minute of exercise at SL ($P = 0.73$) but was significantly lower in AH and CH (both $P < 0.01$). This decrease was significantly greater in AH vs. CH. HHb was unchanged from baseline to warm-up at SL ($P = 0.80$) but decreased significantly in AH and CH. Compared with baseline, HHb was unchanged during the final minute of exercise at SL ($P = 0.24$) but similarly increased in AH and CH (both $P < 0.01$). THb was unchanged from baseline to warm-up in all three conditions. In contrast, compared with baseline, THb was increased significantly and similarly ($P = 0.37$) during the final minute of exercise in all three conditions.

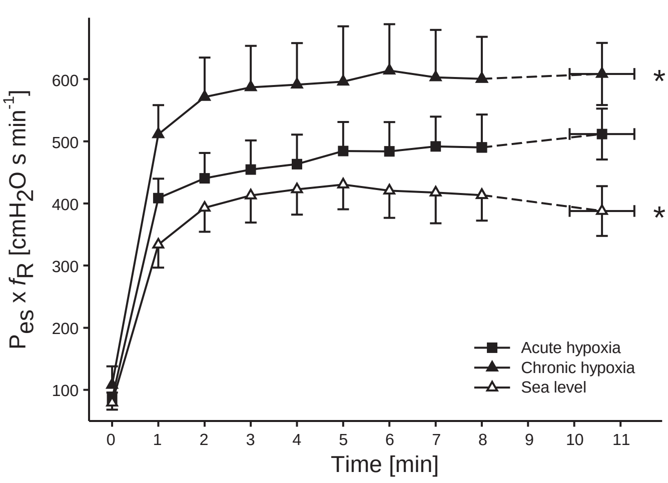

Fig. 1. Inspiratory muscle pressure-time product [esophageal pressure (P_{es}) × respiratory frequency (f_R)] during the identical constant-load cycling exercise performed in all 3 conditions. *$P < 0.05$ vs. acute hypoxia (AH), $n = 8$.

DISCUSSION

The purpose of this investigation was to evaluate the effect of altitude acclimatization on the development of fatigue during whole-body endurance exercise. Subjects repeated the identical constant-load cycling exercise at SL, in AH, and in CH. No measurable degree of fatigue was found following the exercise at SL. However, the identical exercise in AH, characterized by a reduced C_aO_2 and increased W_{insp}, resulted in a substantial degree of both peripheral and central fatigue. Two weeks of exposure to 5,260 m restored C_aO_2 to SL values but increased W_{insp} further over that observed in AH. The critical finding was that the rate of development of peripheral locomotor muscle fatigue failed to recover from AH to CH and was similar in both conditions. In contrast, the development of central fatigue was attenuated significantly in CH (vs. AH) but still greater compared with SL. Taken together, our findings suggest that acclimatization to high altitude attenuates the impact of AH on the development of central fatigue but fails to improve the exacerbated development of peripheral fatigue present during exercise in AH.

Peripheral Fatigue

Acute hypoxia. The cycling bout in AH was, compared with SL, characterized by a substantially exaggerated rate of peripheral fatigue (Table 2 and Fig. 2). These observations confirm numerous earlier findings using whole-body (4, 31, 57) and single-muscle exercise (28, 39).

Table 2. *Effects of constant-load cycling exercise on quadriceps muscle function*

	Percent Change from Pre- to Immediately Postexercise		
	Sea Level	Acute Hypoxia	Chronic Hypoxia
$Q_{tw,pot}$	−3.1 ± 1.8*	−20.9 ± 2.4	−18.8 ± 3.4
MRFD	−4.1 ± 2.5*	−21.2 ± 4.2	−17.9 ± 3.5
MRR	2.7 ± 2.8*	−13.2 ± 3.1	−9.0 ± 2.2
$RT_{0.5}$	1.0 ± 2.2*	9.2 ± 1.3	8.2 ± 1.4
MVC	−1.3 ± 1.2*	−12.3 ± 1.2	−8.9 ± 1.3†
Voluntary muscle activation	−0.1 ± 1.0*	−6.9 ± 1.1	−3.7 ± 1.2†
M-wave amplitude	0.7 ± 2.7*	2.5 ± 2.0*	−7.8 ± 2.1

Changes in muscle function are expressed as a percent change from pre-exercise baseline. All exercise trials were performed for the same duration (10.6 ± 0.7 min) and at the same absolute workload (138 ± 14 W).Values are expressed as means ± SE. $Q_{tw,pot}$, potentiated single twitch; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; $RT_{0.5}$, 1/2 relaxation time; MVC, maximal voluntary contraction force; M-wave, compound muscle action potential. Percent muscle activation is based on superimposed twitch technique. Various variables in acute and chronic hypoxia were, compared with baseline, altered significantly, 2.5 min after exercise ($P < 0.01$). *Not significantly different from pre-exercise baseline. †$P < 0.05$ vs. acute hypoxia, $n = 8$.

Compared with SL, C_aO_2 was approximately one-third lower and W_{insp}, ~34% higher during exercise in AH. These substantial alterations are known to contribute about equally to the exacerbated development of peripheral fatigue in AH (2). The impact of an acutely lowered C_aO_2 on muscle fatigability is mediated via the facilitating effects of the associated reduction in muscle O_2 delivery on the intramuscular accumulation of metabolites known to cause peripheral fatigue, i.e., hydrogen ion and inorganic phosphate (37, 61). The W_{insp}-induced exacerbation of peripheral fatigue results from the same intramuscular metabolic consequences associated with reductions in locomotor muscle O_2 delivery. However, in the case of the W_{insp}-related impairment in peripheral fatigue, the compromised O_2 delivery is the consequence of a sympathetically mediated impact on Q_L, secondary to the activation of the respiratory muscle metaboreflex (34). Taken together, the combined effects of a significantly reduced C_aO_2 and a higher W_{insp} has a profound impact on leg O_2 delivery and thus peripheral locomotor muscle fatigue (1).

Chronic hypoxia. Despite 2 wk of acclimatization to altitude, the rate of development of peripheral locomotor muscle fatigue was similar in AH and CH (Table 2 and Fig. 2). Somewhat conflicting data from earlier investigations suggest different mechanisms as a potential explanation of this finding. On the one hand, studies conducted by Reeves and colleagues (8, 64), following 2–3 wk at 4,300 m, report similar locomotor muscle O_2 delivery during submaximal endurance exercise in AH and CH. Given the critical dependency of the development of peripheral fatigue on muscle O_2 delivery, this similarity might explain the nearly identical levels of end-exercise locomotor muscle fatigue in AH and CH. On the other hand, experiments conducted at the same location as the present study (Mt. Chacaltaya, 5,260 m) have documented a significant improvement in leg muscle O_2 delivery from AH to CH, with the net

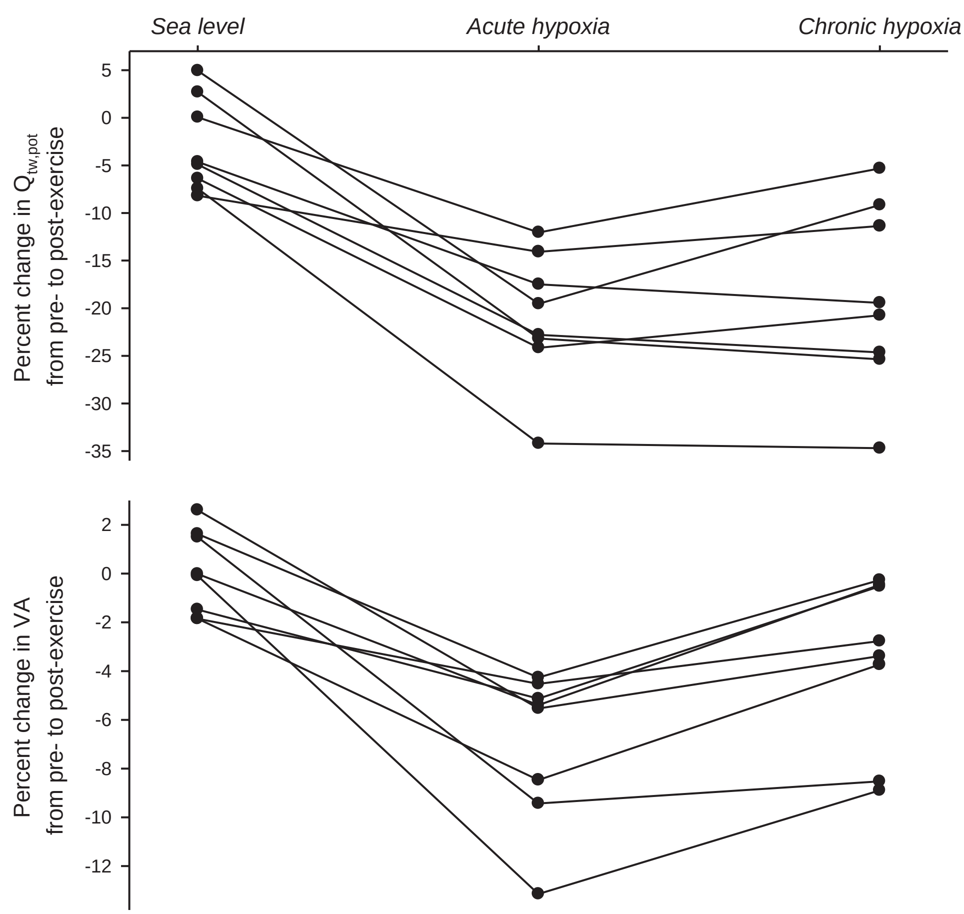

Fig. 2. Individual data illustrating the effects of constant-load bike exercise (138 ± 14 W; 10.6 ± 0.7 min) on potentiated quadriceps twitch force ($Q_{tw,pot}$; *top*) and voluntary muscle activation (VA; *bottom*) at sea level [SL; resting arterial oxygen (O_2) content: 19.3 ± 0.7 ml O_2/dl] and in AH (17.3 ± 0.5 ml O_2/dl) and chronic hypoxia (CH; 20.3 ± 1.3 ml O_2/dl).

effect of similar values during submaximal bike exercise at SL and in CH (13). It might be important to emphasize that these latter experiments involved a greater altitude (5,260 m vs. 4,300 m) and a 9–10 wk acclimatization period vs. only a 2–3 wk period, as in the experiments by Reeves and colleagues (8, 64), as well as the present study. Regardless, based on the findings from the earlier Chacaltaya experiments, it appears that the similar degrees of end-exercise fatigue in AH and CH in the present study (Fig. 2) might have occurred in the face of a significant difference in bulk muscle O_2 delivery, i.e., higher in CH vs. AH.

Q_L was not measured directly in the present study. However, changes in THb, a NIRS-derived variable, are thought to reflect changes in regional blood volume and potentially Q_L (24, 59). The previously documented similarity in resting Q_L at SL, in AH, and in CH (11, 12, 36, 49, 50) is a critical prerequisite when using THb as an estimate of potential differences in Q_L and O_2 delivery during exercise. Since C_aO_2 was comparable at SL and CH (see RESULTS), the same exercise-induced increase in THb (Fig. 3) suggests a similar degree of O_2 delivery in these conditions. Furthermore, the combination of a lower C_aO_2 in AH vs. CH (and SL; see RESULTS) plus the similar increase in THb during exercise (Fig. 3) insinuates a lower locomotor muscle O_2 delivery in AH vs. CH (and by extension, SL). Both of these observations might support earlier blood flow studies conducted at the same location as the present experiments (13) but might contradict others performed at a lower altitude (8, 64). However, NIRS findings obtained from skeletal muscle need to be interpreted with caution. A significant limitation associated with NIRS is that this measurement is confined to a finite location, and changes in THb might not be representative of the whole muscle. Indeed, significant blood flow heterogeneity has been documented previously in skeletal muscle (35). Whereas heterogeneity diminishes with higher exercise intensities and is not affected by hypoxia (36), the exact location of NIRS probe placement from day to day is a potential source of error. To minimize this risk, we had strict criteria regarding probe placement (see METHODS), and at least two investigators independently assured correct probe positioning before each experiment.

Assuming that the similar degrees of peripheral fatigue in AH vs. CH occurred in the face of a greater O_2 delivery in CH, other, rather disadvantageous adaptations associated with acclimatization must have outweighed this benefit. A potential candidate is the documented impairment in the capacity of skeletal muscle to extract O_2 in CH, i.e., a decreased capillary muscle O_2 conductance (41). This impact might, despite a similar O_2 delivery at SL and in CH, potentially lower extracellular PO_2 to or beyond a previously suggested critical value (~30 Torr) associated with exacerbated development of peripheral fatigue (55). Alternatively, the higher O_2 delivery in CH vs. AH (13), combined with the same degree of peripheral fatigue, might suggest that C_aO_2 and bulk O_2 delivery, per se, might not depict key determinants of the exaggerated fatigability in hypoxia. Important here is the fact that despite the normalized C_aO_2 and bulk O_2 delivery in CH, P_aO_2 only partially recovers with acclimatization and remains fairly low in CH. This could hint toward a key role of P_aO_2 in exacerbating the development of peripheral fatigue at altitude.

In CH, V_E was ~20% higher compared with AH. Given the substantially lower air density at 5,260 m (0.64 kg/m^3 vs. 1.18 kg/m^3 at 130 m, where AH experiments occurred), it could be argued that in terms of respiratory muscle work, the reduced density might balance the acclimatization-induced increase in V_E, with the net effect of a similar W_{insp} in CH and AH. However, W_{insp} was, similar to V_E, ~20% higher in CH vs. AH. This observation, per se, might suggest that the lower air density at altitude had no effect on the relationship between minute V_E and respiratory muscle work. However, it has been shown that bronchoconstriction, associated with severe hypoxia, increases the resistive component of respiratory work and offsets the theoretical benefit of a reduced air density (22). This results in a similar respiratory muscle work for a given V_E at altitude and at SL (18). Therefore, any increase in W_{insp}

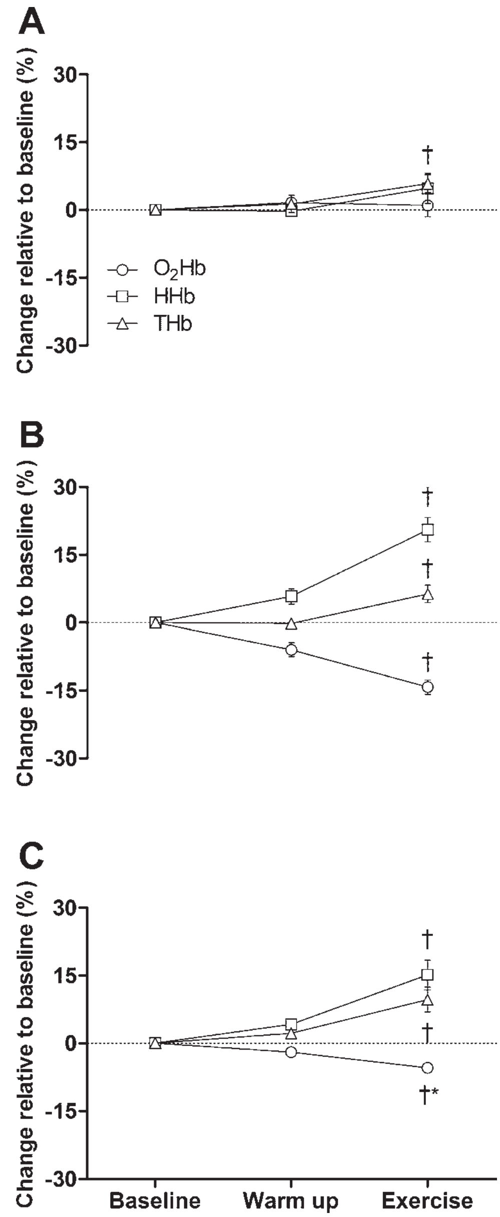

Fig. 3. Vastus lateralis oxygenation at resting baseline, during the final 30 s of a 3-min warm-up (28 W), and during the final 30 s of constant-load exercise (131 W) at SL (*A*), in AH (*B*), and in CH (*C*). †$P < 0.05$ vs. respective baseline; *$P < 0.05$ vs. AH, $n = 8$. O_2Hb, oxyhemoglobin; HHb, deoxyhemoglobin; THb, total hemoglobin.

observed in hypobaric CH is attributable to the exaggerated ventilatory response associated with altitude acclimatization.

The increase in minute V_E in the present study was mainly due to the increase in V_T; f_R was similar in both conditions. The higher V_T was achieved via reductions in EELV (Table 1), which is compared with increasing EILV to raise V_T, more economical, since higher lung volumes are associated with a reduced compliance (38). We therefore conclude that the 23% higher W_{insp} at the same workload in CH vs. AH resulted from the substantially higher V_E following acclimatization. Finally, this exaggerated W_{insp} likely aggravated the respiratory muscle metaboreflex and associated impact on leg vascular conductance (25) and presumably blunted exercise Q_L more in CH compared with AH.

In contrast to our findings, it was suggested previously that acclimatization to high altitude might eliminate the impact of AH on the rate of development of fatigue during single muscle exercise (adductor pollicis) and restore it to that observed at SL (28). However, submaximal, intermittent exercise, including a small muscle mass, does not maximally challenge O_2 delivery and use. Therefore, the observed positive effect could, at least in part, be explained by the use of the available reserve capacity. Specifically, various compensatory mechanisms, including increases in cardiac output and muscle O_2 delivery and extraction, could have reduced the hypoxia-induced impact on the development of fatigue. Such an effective compensation might not—or only to a much smaller degree–be possible during intense, whole-body exercise, performed close to a human's maximal circulatory and ventilatory capacity (14, 15).

CH had a significant impact on the effect of exercise on M-wave amplitude. Reductions in M-wave amplitude have been associated with decreases in sarcolemma excitability (19). The attenuated excitability results from reduced sarcolemma sodium (Na^+)-potassium (K^+)-ATPase activity (46) and can contribute to compromised muscle force output (21). Pre-exercise M-wave amplitudes (and $Q_{tw,pot}$) in our experiments were similar in all three conditions. This suggests that neither severe AH nor CH impairs sarcolemma Na^+-K^+-ATPase activity and membrane excitability of resting locomotor muscle. This confirms earlier findings (40); however, it contrasts with others (16) who report decreased resting M-wave amplitudes following 10 days of exposure to severe hypoxia (>4,300 m). Regardless, although M-wave amplitudes did not change from pre- to postexercise at SL and in AH, we observed, in contrast to Garner et al. (30), a significant exercise-induced decrease in CH (Table 2). AH has recently been shown to have no effect on exercise-induced changes in Na^+-K^+-ATPase activity, which explains the similar M-wave behavior in SL and AH (51). However, altitude acclimatization causes a downregulation of Na^+-K^+-ATPase pump concentration, and although this does not alter resting M-wave characteristics, it likely explains the exercise-induced decrease in M-wave amplitude observed in CH (20, 33).

The lower postexercise M-wave amplitude in CH indicates a failure of the motor nerve/sarcolemma to propagate evoked stimuli to the contractile apparatus and might have masked potential benefits of acclimatization on fatigue resistance. Put simply, postexercise twitch forces might have been larger in CH if M-waves had remained unchanged from pre-exercise. If so, this would have resulted in a smaller exercise-induced reduction in $Q_{tw,pot}$ in CH. Regardless, failure of neuromuscular transmission/sarcolemmal excitability contributes to reduced force output in response to a given central nervous activation and can therefore be considered a key determinant of the impaired fatigue resistance in CH.

Central Fatigue

Exercise in AH induced a substantial degree of central fatigue, which was attenuated by ~50% when the same trial was repeated in CH (Table 2). This significant improvement, associated with acclimatization, clearly contrasts with the absence of a beneficial effect of CH on peripheral fatigue, as described above. Since the development of central fatigue is highly sensitive to O_2 (1), we attribute this improvement to the effects of high-altitude acclimatization on O_2 availability within the brain. Specifically, the cerebral O_2 delivery index at the end of exercise in CH was improved from AH (Table 1) (65) and may explain the lower degree of central fatigue in CH vs. AH.

Despite the similar CBFv and a slightly higher brain O_2 delivery in CH vs. SL (Table 1), which agrees with earlier Chacaltaya studies using the Kety-Schmidt technique to measure CBF/O_2 delivery (44), exercise-induced central fatigue was greater in CH. Two considerations discussed previously might account for this observation. First, the significant degree of peripheral fatigue in CH (vs. no fatigue at SL) presumably facilitated central fatigue via increases in inhibitory neural feedback from locomotor muscle (mediated by *group III/IV* muscle afferents), which limit central motor drive (3, 6). Second, although C_aO_2 and brain O_2 delivery were similar/higher in CH vs. SL, the still substantially lower P_aO_2 might have contributed to the greater degree of central fatigue during exercise in this condition. Indeed, a low P_aO_2 was recently suggested to impair cerebral metabolism (48) and alterations in neurotransmitter turnover (23), and both of these factors have been linked to the development of central fatigue (17, 53).

Taken together, the current findings provide a global indication of the positive effects of altitude acclimatization on the development of central fatigue during exercise. However, we cannot comment on the specific sites of the central motor pathway involved or the relative contribution of C_aO_2 and P_aO_2 in mediating these beneficial adaptations.

Implications of Findings for Performance-Related Questions in CH

AH generally impairs endurance exercise performance (60). Prolonged exposure to hypoxia is known to recover some of this impairment (29, 52); however, SL performance is never matched at altitude. Our current findings indicate that the acclimatization-induced partial recovery of endurance performance occurs independent of any improvement of peripheral locomotor muscle fatigue from AH to CH. This insinuates that peripheral locomotor muscle fatigability, per se, does not contribute to the improvement of endurance performance observed from AH to CH. We therefore propose that the significantly attenuated central fatigue during exercise in severe CH likely accounts, at least in part, for the improvement of endurance performance associated with altitude acclimatization.

Mechanisms underlying the hypoxia-induced curtailment of central motor drive (i.e., increase in central fatigue) and endurance exercise performance have been documented previously to differ depending on the severity of arterial hypoxemia. Specifically, peripheral fatigue might depict the dominant determinant of central motor drive and thus the limiting factor

above 70–75% S_pO_2. At more severe degrees of hypoxemia (<70% S_pO_2), central motor drive and endurance performance might primarily—but not exclusively—be determined/limited by central nervous system (CNS) hypoxia (5). Since peripheral fatigue did not change with acclimatization in the present study, but S_pO_2 increased from below to above the "threshold" described previously (5), reductions in central fatigue might be mediated mainly by improved arterial oxygenation and associated smaller influence of CNS hypoxia on central motor drive.

A recent Point:Counterpoint debate in this journal has focused on the potential existence/relevance of differences in physiological responses to exercise performed in normobaric vs. hypobaric hypoxia (43, 45). Since the present AH and CH experiments were performed in normobaric and hypobaric hypoxia, respectively, these potential differences, if indeed existent, might have influenced our findings.

Conclusion

AH exacerbates central and peripheral fatigue during endurance exercise. Our experiments indicate that acclimatization to high altitude significantly attenuates the development of central fatigue but does not improve the development of peripheral fatigue observed during whole-body endurance exercise in AH.

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DISCLOSURES

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Author contributions: M.A., S.G., and A.W.S. conception and design of research; M.A., S.G., R.T., and A.W.S. performed experiments; M.A., S.G., and R.T. analyzed data; M.A., S.G., R.T., and A.W.S. interpreted results of experiments; M.A. and S.G prepared figures; M.A. and A.W.S. drafted manuscript; M.A., S.G., R.T., A.W.S., A.T.L., and R.C.R. edited and revised manuscript; M.A., S.G., R.T., A.W.S., A.T.L., and R.C.R. approved final version of manuscript.

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AltitudeOmics: Decreased reaction time after high altitude cognitive testing is a sensitive metric of hypoxic impairment

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Humans experiencing hypoxic conditions exhibit multiple signs of cognitive impairment, and high altitude expeditions may be undermined by abrupt degradation in mental performance. Therefore, the development of psychometric tools to quickly and accurately assess cognitive impairment is of great importance in aiding medical decision-making in the field, particularly in situations where symptoms may not be readily recognized. The present study used the Defense Automated Neurobehavioral Assessment (DANA), a ruggedized and portable neurocognitive assessment tool, to examine cognitive function in healthy human volunteers at sea level, immediately after ascending to an elevation over 5000 m, and following 16 days of acclimatization to this high altitude. The DANA battery begins with a simple reaction time test (SRT1) which is followed by a 20-min series of complex cognitive tests and ends with a second test of simple reaction time (SRT2). Tabulating the performance scores from these two tests allows the calculation of an SRT change score (dSRT = SRT1 - SRT2) that reflects the potential effect of mental effort spent during the 20-min testing session. We found that dSRT, but not direct SRT in comparison to sea-level baseline performance, is highly sensitive to acute altitude-related performance deficits and the remission of impairment following successful acclimatization. Our results suggest that dSRT is a potentially useful analytical method to enhance the sensitivity of neurocognitive assessment. *NeuroReport* 00:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The partial pressure of oxygen reduces exponentially with increasing altitude and leads to hypoxia, an underlying cause of cognitive and physiological impairment at high altitude. In general, the severity of impairment is a function of both altitude and the rate of ascent, where moderate altitudes (< 2000 m) and slow elevation gains induce little decrement compared with extreme altitudes (> 6000 m) and rapid ascension, which have more severe effects and can result in loss of consciousness or death [1].

Rapid ascent to high altitude results in a number of impairments in cognitive performance (see [2] for review), although it should be noted that fatigue and other travel-related factors must be accounted for before attributing impairment to hypoxia alone [3]. Impairments due to hypoxia have been observed in short-term memory [4], long-term memory and verbal expression [5], attention [6,7], and reaction time [8,9]. Because of the potential impact of these impairments upon high altitude expeditions, the development of field-deployable tools to aid the assessment of hypoxia-induced cognitive impairment is highly relevant to medical decision-making in these scenarios.

In this report, we analyzed an unexamined feature of the neurocognitive data collected from healthy human volunteers on an expedition to Mt Chacaltaya in Bolivia [10]. Cognitive performance was assessed in this study using the Defense Automated Neurobehavioral Assessment (DANA), a software package of public domain cognitive tests that runs on the Android platform. DANA was originally developed as a means of rapidly assessing cognitive changes following mild traumatic brain injury/concussion in deployed service members exposed to blasts, and its reliability has been previously validated in a number of extreme environments [11]. The DANA test battery includes two administrations of a simple reaction time (SRT) task: one at the beginning and one at the end of the ∼20-min test session. To investigate the hypothesis that the second measurement of reaction time might reveal an effect of mental fatigue on cognitive performance, we tabulated a dSRT score to compare throughput, a measure of cognitive efficiency, between the two reaction time administrations. Here we show that performance decreases across DANA testing as a function of acute exposure to hypoxic conditions, an

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altitude-related decrement that resolves following successful physiological acclimatization to high altitude.

Methods

Volunteer subjects

As part of the AltitudeOmics study on the physiological signatures of altitude acclimatization [10], DANA was administered to a group of volunteers at sea level (SL) and at 5260 m atop Mt Chacaltaya near La Paz, Bolivia. The study was performed according to the Declaration of Helsinki and was approved by the Institutional Review Boards of the University of Colorado and the University of Oregon, as well as the Human Research Protection Office of the US Department of Defense. The detailed methods for the overall study are summarized here and described elsewhere [10]. Before giving written and verbal consent to participate, each volunteer was informed of the possible risk and discomforts involved in the study. From a pool of 79 volunteers, a total of 24 were recruited under strict criteria including birth at a low elevation (< 1500 m), physical fitness, and general health characteristics (not pregnant or lactating, no prescription drug use, and no history of migraine, loss of consciousness, smoking, cardiovascular abnormality, or pulmonary dysfunction). Of the recruited participants, three dropped out of the study because of medical reasons apart from altitude sickness (e.g. gastrointestinal illness), resulting in a total of 21 participants (12 male, nine female; mean age 20.8 years, range 19–23 years). The constraints of the study, including strict inclusion/exclusion criteria, travel costs, and subject travel availability, produced a small and relatively homogenous sample.

The experiment proceeded according to the following timeline: first, the participants underwent baseline testing at SL (Eugene, Oregon, USA) ∼1 month before traveling to Bolivia. After an overnight flight to El Alto (4050 m), the participants immediately descended to Corocio (1525 m) where they rested for 48 h to limit the effects of jet lag. Next, pairs of participants were driven to the top of Mt Chacaltaya (5260 m) over a period of 3 h. During the drive, supplemental oxygen was provided to each participant through either a mask or a nasal cannula (2 l/min) to allow an assessment of acute change upon reaching the destination altitude. After the ascent, one member of each pair immediately began testing, whereas the other continued to breathe supplemental oxygen for 2 h until his/her turn for testing. The assessment was repeated after 16 days of acclimatization at 5260 m, which included descents to La Paz (3800 m) over the first 4 days. A final round of sea-level (SL) testing was conducted ∼3 months after returning from Bolivia to collect data from participants who missed the initial SL testing.

DANA administration

The DANA test battery includes the following tests: SRT1, code substitution (simultaneous), procedural reaction time, spatial discrimination, go/no-go, code substitution (delayed), match to sample, and Sternberg memory search. The ∼20-min test battery ended with a second administration of simple reaction time (SRT2) according to methodology introduced by Bleiberg *et al.* [12]. The test battery was administered on a Trimble Nomad handheld computer (Android version 2.1; Trimble, Sunnyvale, California, USA). Performance in the tests administered between SRT1 and SRT2 was analyzed elsewhere [10]; for the present purposes, the intervening test battery between the two SRT administrations may be thought of as providing a cognitive challenge to the participant, which we hypothesized to have a negligible effect upon SRT2 under normal conditions.

Each SRT administration consisted of 40 trials with a random intertrial interval (600–3000 ms). Each trial began when a yellow target appeared on a black screen. The participant was instructed to tap the target as soon as it appeared and was asked to perform the task as quickly and accurately as possible. The participants completed four practice trials with feedback before commencing the portion of the test from which data were collected.

Data analysis

Throughput was calculated as follows for each SRT administration per participant:

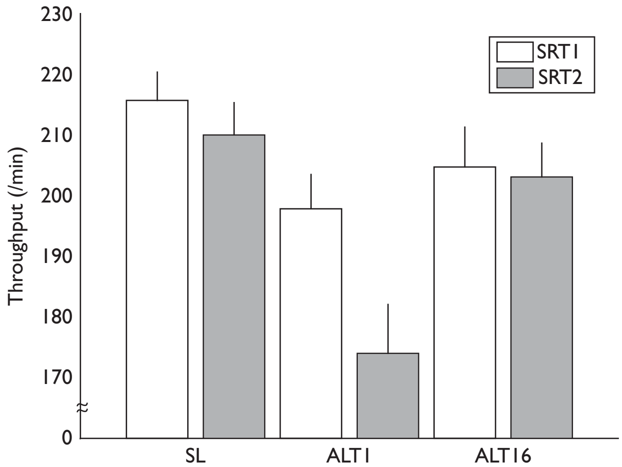
$$\text{Throughput} = \frac{\text{Correct trials (\%)}}{\text{Correct trial median reaction time (ms)}} \times 60000 \left(\frac{\text{ms}}{\text{min}}\right)$$

If the percentage of incorrect trials (i.e. failing to respond within 900 ms or responding in anticipation of the cue) exceeded 33% for any SRT administration, whether because of suboptimal effort, illness, or sleep deprivation, the participant was excluded from the analysis ($n = 2$). A two-way, repeated measures analysis of variance was used to analyze global effects on throughput. Pairwise comparisons were examined using Bonferroni-corrected paired t-tests (significance at $P < 0.05/4 = 0.0125$), and effect sizes were calculated with Cohen's d. Because the average throughput from the two SL administrations was not significantly different (unpaired t-test, $P = 0.51$), the second administration was used for the baseline comparison as it included complete datasets from all of the participants. All analyses were carried out using MATLAB R2013b (Mathworks, Natick, Massachusetts, USA).

Results

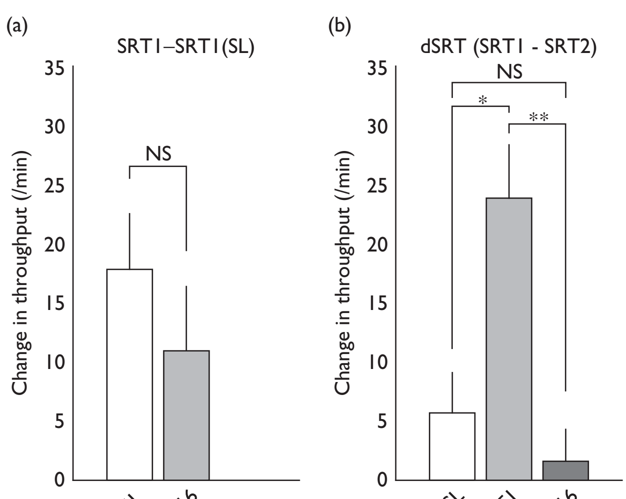
Participant performance in a simple reaction time test was assessed at the beginning (SRT1) and end (SRT2) of a 20-min DANA testing session at SL, following an ascent to 5260 m (ALT1), and after 16 days of acclimatization to this altitude (ALT16; Fig. 1). A two-way, repeated measures analysis of variance with the factors altitude (SL, ALT1, or ALT16) and administration (SRT1 or SRT2) revealed significant main effects of altitude ($F = 15.96$, $P < 0.0001$)

Fig. 1



Throughput in a simple reaction time (SRT) task as a measure of cognitive performance during acute altitude exposure, after acclimatization, and at sea level (SL). SRT was administered before (SRT1) and after (SRT2) cognitive loading with DANA testing. Following acute altitude exposure (ALT1), both SRT1 and SRT2 performance decreased, with a particular decrement in the second administration. Following two weeks of acclimatization (ALT16), performance in the SRT task approximated sea level (SL) scores. Error bars represent SE from the mean. DANA, Defense Automated Neurobehavioral Assessment.

Fig. 2



Quantification of altitude and exertion-related performance changes. (a) Simple reaction time (SRT) performance during acute altitude exposure (ALT1) and following two weeks of acclimatization (ALT16) was compared with sea-level (SL) performance. There was no significant difference between ALT1 and ALT16 when compared with SL throughput. (b) Change in SRT performance across DANA testing was compared for each time point. A marked decrease in throughput was observed at ALT1, whereas ALT16 showed an indistinguishable difference from SL. *$P<0.005$, **$P<0.001$. DANA, Defense Automated Neurobehavioral Assessment.

and administration ($F=22.02$, $P<0.0005$), as well as a significant interaction of these terms ($F=11.37$, $P<0.0005$) upon SRT throughput.

Post-hoc testing detected no difference between SRT1 and SRT2 throughput at SL ($P=0.43$). This finding was corroborated by additional analysis of a previously collected dataset [11], in which a similar, ~15-min version of DANA was administered to groups of healthy volunteers in a variety of extreme climates (desert, jungle, aboard a ship, and at high altitude postacclimatization). Paired *t*-tests comparing throughput in SRT1 versus SRT2 failed to reach significance in any of these climates (all P's >0.05), supporting the conclusion that the intervening tests in the DANA battery do not adversely impact SRT performance in healthy humans under these conditions.

To quantify the apparent altitude-induced impairment in reaction time, we first examined SRT performance by comparing against baseline values, a method favored by many neurocognitive assessment protocols [13,14]. Each participant's SL 'baseline' SRT1 throughput was subtracted from the SRT1 throughput values at ALT1 and ALT16. On average, participants showed a throughput decrease of 17.85 ± 4.71/min (mean ± SE) at ALT1 and 10.97 ± 5.45/min at ALT16 compared with SL baseline. A paired *t*-test revealed that this baseline comparison measure failed to show a significant difference between ALT1 and ALT16 ($P=0.24$; Fig. 2a).

A second comparison, dSRT, was calculated as the difference in each participant's SRT1 and SRT2 throughput scores at each time point (SL, ALT1, and ALT16). These comparisons revealed that SRT2 throughput decreased at the end of DANA testing by an average of 23.87 ± 4.52/min at ALT1, 1.63 ± 2.71/min at ALT16, and 5.71 ± 3.42/min at SL, showing a significant difference between ALT1 and ALT16 ($P<0.001$) and between ALT1 and SL ($P<0.005$; Fig. 2b). In addition, the dSRT comparisons produced much larger effect sizes than the baseline comparison (dSRT ALT1 vs. ALT16 $d=0.95$, ALT1 vs. SL $d=0.75$; baseline $d=0.28$).

Discussion

In accordance with previously collected evidence from healthy human volunteers [11], no difference was detected between SRT1 and SRT2 throughput at SL. These results support the hypothesis that DANA testing does not induce sufficient cognitive loading to alter psychomotor performance in healthy participants under normal inspired oxygen and barometric pressure. However, a comparison of performance in the two SRT administrations unmasked a robust altitude-dependent effect of cognitive exertion upon psychomotor efficiency. The difference score dSRT (SRT1 throughput − SRT2 throughput) shows a significant relationship with acute altitude exposure: a marked decrease in throughput

following cognitive testing emerges after ascent from SL. However, following 16 days of acclimatization to high altitude, throughput scores resemble those seen at SL: SRT1 and SRT2 performances are indistinguishable. These results are in agreement with complementary physiological and cognitive data that were simultaneously collected from the same participants [10]. In contrast, the baseline comparison measure failed to show a significant difference between ALT1 and ALT16. These results indicate that in this context, comparison against baseline was not sensitive to the cognitive effects of acute hypoxia and subsequent acclimatization. Further, the dSRT comparison produced a much larger effect size than the baseline comparison, indicating that dSRT is a robust metric by which cognitive impairment may be quantitatively assessed.

A similar post-testing decrease in reaction time was reported by Bleiberg *et al.* [15] in a study on fatigue in postpolio patients. In this study, an Automated Neuropsychological Assessment Metrics (ANAM) battery [12] was used with a configuration similar to DANA: an SRT task was presented both at the beginning and at the end of a battery of more complex cognitive tests. The participants began the morning with a complete ANAM battery, underwent a 1-h comprehensive functional medical evaluation including motor testing and other fatiguing activities, and then completed a second round of the ANAM battery. Although less than a quarter of the postpolio participants showed a decrement in SRT1, over 50% showed decreased performance in SRT2, a difference which was highly statistically significant. Together with the present results, these data indicate that performance in an SRT task after cognitive loading may be a highly sensitive means for observing cognitive impairment. However, the parameters (e.g. length, difficulty, repetition, etc.) of the testing battery that are responsible for the observed results are yet to be identified. It could be the case that a more condensed assessment may be sufficient to reveal cognitive impairment; alternatively, greater sensitivity to impairment may be achievable using an optimized test battery.

Although the observed decrement in SRT performance upon acute hypoxia exposure could be interpreted as motor fatigue rather than cognitive impairment *per se*, we note that several of the other reaction time tasks interleaved within the test battery did not show a significant difference between SL and ALT1 [10]. Taken together, these results indicate that decreased motor output alone cannot explain the change in performance; however, more research is required to investigate the complex interaction of cognitive and motor processing under these conditions.

Conclusion

Comparing SRT performance at the beginning and end of a DANA test battery provides a more robust and reliable indication of hypoxia-induced cognitive impairment than the typically used comparison against baseline performance. Because SRT throughput does not decrease across testing under normal conditions, these results suggest that calculating the dSRT score is a promising analytical method that may aid neurocognitive assessment in situations where appropriate baseline data are not available.

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This paper is one in a series titled 'AltitudeOmics' that together represents a group of studies exploring the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations invested enormous time and resources to make this project a success. Foremost, the study was made possible by the tireless support, generosity, and tenacity of our research participants (please see Subudhi *et al.* [10] for a complete list of people and organizations who contributed to this effort). In addition, the authors would like to thank Lindsay Long for her assistance in organizing the data and James Drane, Julia Kern, and Sonja Jameson-Van Houten for their assistance with data collection. Finally, the authors are grateful for the helpful comments and discussion of the manuscript by James Drane, Clementina Russo, and James Spira.

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Conflicts of interest

Emma B. Roach, Corinna E. Lathan, and Lawrence Wolpert are employed by AnthroTronix Incorporated, developer of the DANA tool.

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AltitudeOmics: Rapid Hemoglobin Mass Alterations with Early Acclimatization to and De-Acclimatization from 5260 m in Healthy Humans

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Abstract

It is classically thought that increases in hemoglobin mass (Hbmass) take several weeks to develop upon ascent to high altitude and are lost gradually following descent. However, the early time course of these erythropoietic adaptations has not been thoroughly investigated and data are lacking at elevations greater than 5000 m, where the hypoxic stimulus is dramatically increased. As part of the AltitudeOmics project, we examined Hbmass in healthy men and women at sea level (SL) and 5260 m following 1, 7, and 16 days of high altitude exposure (ALT1/ALT7/ALT16). Subjects were also studied upon return to 5260 m following descent to 1525 m for either 7 or 21 days. Compared to SL, absolute Hbmass was not different at ALT1 but increased by 3.7±5.8% (mean ± SD; n = 20; $p<0.01$) at ALT7 and 7.6±6.6% (n = 21; $p<0.001$) at ALT16. Following descent to 1525 m, Hbmass was reduced compared to ALT16 (−6.0±3.7%; n = 20; p = 0.001) and not different compared to SL, with no difference in the loss in Hbmass between groups that descended for 7 (−6.3±3.0%; n = 13) versus 21 days (−5.7±5.0; n = 7). The loss in Hbmass following 7 days at 1525 m was correlated with an increase in serum ferritin (r = −0.64; n = 13; $p<0.05$), suggesting increased red blood cell destruction. Our novel findings demonstrate that Hbmass increases within 7 days of ascent to 5260 m but that the altitude-induced Hbmass adaptation is lost within 7 days of descent to 1525 m. The rapid time course of these adaptations contrasts with the classical dogma, suggesting the need to further examine mechanisms responsible for Hbmass adaptations in response to severe hypoxia.

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Introduction

Precise regulation of erythropoiesis is critical, as both anemia and excessive polycythemia have detrimental effects on physiological function. Hypoxia is a potent stimulator of erythropoiesis and erythropoietin (EPO) increases within hours of hypobaric hypoxia [1]. However, it is classically thought that elevations in total hemoglobin mass (Hbmass) and red cell volume (RCV) during high altitude acclimatization require several weeks to occur [2,3]. This delayed increase fits with patterns observed with exogenous EPO administration in healthy humans, where Hbmass/RCV have been consistently reported to remain unchanged within the first 12 days of treatment [4,5,6]. Although previous studies examining erythropoeitic adaptations in lowlanders adapting to altitudes greater than 4000 m for periods longer than 4 weeks have consistently reported increases in Hbmass/RCV [7,8], the time course of erythropoietic adaptation during early (i.e., first 1–3 weeks) high altitude acclimatization is less clear. For example, whereas some studies have found unchanged RCV following 2–3 weeks at 4300 m [9,10], others have found moderate increases at this same elevation over a similar time course [11,12]. More recently, studies examining the early time course of changes in Hbmass at lower altitudes (2000 m–3600 m) have reported small (2–3%) but significant increases in Hbmass following 11–13 days [13,14,15]. Data from these studies conflict with the classical dogma that at least 3–4 weeks are required for increases in Hbmass to be observed, but this remains a matter of considerable debate [16,17]. Further investigation of early erythropoietic adaptations to high altitude is warranted and, importantly, data are lacking at elevations greater than 5000 m where the hypoxic stimulus for erythropoiesis is dramatically increased [18,19].

Gains in Hbmass/RCV obtained during high altitude acclimatization are eventually lost following descent to low altitude, but

the time course of this de-acclimatization also remains unclear. Based upon the traditional kinetics of red blood cell production and destruction, large reductions in Hbmass/RCV are expected to take multiple weeks to occur. Recent altitude training studies conducted at elevations between 2000 m–3600 m have reported full or partial retention of altitude-induced gains in Hbmass for 2–3 weeks following descent to sea level [14,15,17], with Hbmass eventually returning to baseline sea level values [14,17]. Hbmass/RCV have also been reported to remain elevated for several weeks following cessation of EPO treatment [5,6,20]. These studies suggest that elevations in Hbmass induced with short-term environmental or pharmacologic perturbation decay gradually over several weeks. In contrast, a study of polycythemic high altitude natives from 4380 m reported a rapid loss in RCV of ~7% within the first week of descent to sea level; this rapid loss in RCV was coupled with increases in several markers of red blood cell destruction [21]. Although this study suggested that an increased rate of red blood cell destruction may cause a rapid reduction in Hbmass following high altitude descent, the high altitude natives studied were severely polycythemic and therefore these results may not extend to subjects with less marked polycythemia. As with studies examining changes in Hbmass during the early phase of acclimatization to altitudes greater than 5000 m, we are unaware of any studies examining the early time course of loss in Hbmass following descent from altitudes greater than 5000 m.

AltitudeOmics was designed as a large collaborative research project examining early high altitude acclimatization/de-acclimatization in multiple physiological systems [22]. As a result of this overall project design, we had the unique opportunity to examine the early time course of erythropoietic adaptations with ascent to and descent from 5260 m in healthy humans. Because rapid changes in plasma volume (PV) occur within the first days of high altitude ascent (i.e., [23,24,25,26,27] and descent [26,27], early alterations in hemoglobin concentration ([Hb]) or hematocrit (Hct) do not necessarily reflect changes in Hbmass/RCV. Therefore, we measured Hbmass and blood volume compartments in lowlanders at sea level, on 3 occasions at 5260 m during 16 days of high altitude exposure, and upon initial return to 5260 m following descent to low altitude (1525 m) for either 7 or 21 days.

Methods

Study Design

A detailed description of the overall AltitudeOmics study design and subject characteristics is reported elsewhere [22]. The study was approved by the Institutional Review Boards of the Universities of Colorado and Oregon, and by the Human Research Protection Office of the U.S. Department of Defense. Subjects were informed about the possible risks and discomforts involved before giving written and verbal consent to participate.

The data reported here are novel with the exception of the basic characteristics of the 21 AltitudeOmics subjects (12 males, 9 females; age: 21±1 years; height: 176±8 cm; body mass: 70±9 kg) that have been reported previously [22]. Subjects were studied near sea level (130 m; Eugene, OR, USA) and on 4 occasions at 5260 m (Mt. Chacaltaya, Bolivia). Hbmass/BV compartments were measured in duplicate at sea level (SL) during baseline testing, with the mean of 2 tests used as the SL value. Nine to ten weeks after SL testing, subjects were flown via commercial aircraft to El Alto, Bolivia (4050 m) and then immediately driven to 1525 m (Coroico, Bolivia), where they stayed for 2 days. Subjects were then driven to 5260 m on ALT1 and Hbmass/BV parameters were assessed after 9–13 hours at this elevation. Subjects spent days ALT2-ALT4 at 3800 m (La Paz, Bolivia), with a short visit to 5260 m on ALT4, before returning to 5260 m on ALT5. Subjects remained at 5260 m from ALT5 to ALT17, and Hbmass and BV compartments were assessed on ALT7 and ALT16. On ALT17, subjects descended to 1525 m for either 7 (POST7; n = 14) or 21 days (POST21; n = 7). Subjects were transported back to 5260 m on POST7/POST21 and Hbmass/BV measurements were taken following 9–13 hours at this elevation.

Serum ferritin was assessed in all subjects 2–3 weeks prior to baseline testing. All male subjects had initial ferritin levels greater than 20 ng mL^{-1} and none received iron supplementation during the study. Women with initial ferritin levels less than 20 ng mL^{-1} (n = 7) were directed to take oral iron supplements (325 mg ferrous sulfate, 2–3 times daily) for 2–3 weeks prior to baseline testing and until departure to high altitude. One subject ceased supplementation prior to departure to high altitude due to gastrointestinal complaints. No subjects received iron supplementation following departure from SL. The decision not to provide iron supplementation during high altitude acclimatization/de-acclimatization was made based on potential confounding influences [28,29] of iron supplementation on other physiological responses that were assessed as part of the overall AltitudeOmics project.

Subjects participated in many studies as part of the AltitudeOmics project and some involved blood sampling. At SL, Hbmass/BV assessments were performed prior to any other blood sampling. At high altitude, Hbmass/BV parameters were measured following other blood sampling. The estimated volume of blood withdrawn for sampling at each altitude time point was as follows–ALT1: 212±81 mL; ALT7: 64±26 mL; ALT16: 191±10 mL; POST7/21: 147±46 mL. To examine the effect of blood sampling on Hbmass measured at ALT1, we compared our measured Hbmass values at ALT1 and SL and found that the mean values were not significantly different (see Results). Additionally, when we examined Hbmass across all time points using measured or adjusted (for estimated Hbmass withdrawn due to blood sampling), the statistical significance of our findings remained unaltered. Therefore, we have chosen to report the measured Hbmass values without adjusting for blood withdrawn for sampling but address the magnitude of Hbmass lost due to sampling in the discussion.

Analytical Methods

Hbmass and BV Parameters. Hbmass was measured using the optimized carbon monoxide (CO) rebreathing method [30,31] with minor modifications. Following at least 20 minutes of seated rest, a venous (v) blood sample (~2 mL) was obtained from an antecubital vein and used for determination of v[Hb] (OSM3 hemoximeter, Radiometer, Denmark) and vHct (microcentrifugation). The OSM3 was calibrated for [Hb] at regular intervals according to the manufacturers' recommendations. v[Hb] and vHct were analyzed in triplicate. Arterialized capillary blood samples (200 µL) were obtained from a hyperemic earlobe and measured for baseline carboxyhemoglobin (HbCO%) in sextuplicate on the OSM3. End-tidal [CO] was measured using a portable CO detector (Draeger Pac 7000, Draeger, Germany). Subsequently, a bolus of 99.9% CO was administered to subjects from a calibrated syringe into a custom-built spirometer (Spico-CO Respirations-Applikator, Blood Tec, Germany) and rebreathed for 2 minutes along with 3 to 5 L of 100% oxygen.

The volume of CO administered to subjects was chosen to induce a ~5–6% increase in HbCO%. The volume of CO administered was increased at high altitude based on the reduced barometric pressure to obtain similar ΔHbCO% in tests at sea

level and high altitude. However, the largest volume of CO administered was 135 mL (maximal volume of calibrated syringe). The mean ΔHbCO% following the rebreathing procedure was 5.4±0.8%.

Potential CO leaks from the subject or rebreathing apparatus were monitored throughout the rebreathing procedure using 2 portable CO detectors. Due to the effects of CO leaks on measurement error of Hbmass [32], any test in which a CO leak was detected was excluded (total of 6 tests). End-tidal [CO] was measured 4 minutes following initial CO inhalation. Arterialized capillary blood samples (100 μL) were obtained at minutes 6 and 8 following CO inhalation and analyzed in triplicate, with the mean of minute 6 and 8 taken as post-rebreathing HbCO%. The amount of CO remaining in the spirometer was measured using a calibrated syringe and a portable CO detector (Draeger Pac 7000, Draeger, Germany). All data were compiled and used to calculate Hbmass according to previously published formulas [30,31].

The altitude in the present study was higher than any previous studies employing the optimized CO rebreathing method. To address a potential issue due to differences in oxygen saturation [33] between sea level and high altitude testing conditions, the following minor modification was made. For tests at sea level, subjects breathed a hyperoxic gas mixture (50.5% O_2, balance nitrogen, $P_IO_2 \approx 360$) for 10 minutes prior to baseline blood sampling and throughout the rest of the procedures, with the exception of the 2-minute CO rebreathing procedure, where 100% O_2 was rebreathed along with CO. At high altitude ($P_B = 408$ mmHg), subjects breathed 100% O_2 ($P_IO_2 \approx 360$) for 10 minutes prior to the baseline blood sampling and throughout the rest of the procedures. Hyperoxia was provided at both sea level and high altitude to establish similarly high oxygen saturation levels during the Hbmass procedure in both environments in order to eliminate the influence of oxygen saturation differences on the analytical determination of HbCO% on the OSM3 hemoximeter [33].

A single OSM3 unit was used for all HbCO% measurements during the study. To determine any potential confounding effects of the international transport on the OSM3 hemoximeter or high altitude *per se* on the measurement of HbCO%, we performed a quality control analysis using in-house arterial blood samples that we had obtained prior to this study at 2 levels of HbCO% (high and low HbCO%). These control samples were run in sextuplicate on 5 days during sea level testing and the mean ΔHbCO% between days was 6.84±0.05%; control samples were kept frozen at −80°C until transport–they remained stored on ice during transport and were analyzed in sextuplicate on 2 days within the first week at high altitude. The mean ΔHbCO% between these days was 6.80±0.07%. Thus, the difference in ΔHbCO% between sea level and high altitude was well within the intra-analyzer variability of ΔHbCO% using OSM3 hemoximeters [34], indicating that neither high altitude nor the international transport of the OSM3 had any confounding effects on the measurement of ΔHbCO% that is critical to Hbmass determination.

Measurement errors of Hbmass and BV parameters were calculated from duplicate baseline measurements (n = 19) according to Hopkins [35]. Measurement error for Hbmass was 1.5% (95%CI: 1.1–2.3%)–with duplicate Hbmass tests within a 1-week period, measurement error reflects primarily analytical error as the biological variation over this time frame has been shown to be minimal [36,37].

RCV, BV, and PV were derived from Hbmass, v[Hb], and vHct as follows:

1) $RCV = Hbmass \times MCHC^{-1} \times 100^{-1}$

2) $BV = RCV \times 100 \times Hct^{-1} \times 0.91^{-1}$

3) $PV = BV - RCV$

Hct was multiplied by 0.96 to account for trapped plasma; the constant of 0.91 was included in the BV calculation to correct for the ratio of body hematocrit to peripheral hematocrit [38]. Measurement errors for RCV, PV, and BV were 2.2% (95%CI: 1.6–3.2%), 4.9% (95%CI: 3.6–7.3%), and 3.4% (95%CI: 2.5–5.0%), respectively. For BV compartments, the measurement error is influenced by analytical error of Hbmass, [Hb], and Hct as well as biological variation in PV and total BV.

Serum Ferritin and EPO. Whole blood samples were collected following 30 min of rest from a catheter placed in an antecubital vein [22]. The ALT1 ferritin sample was taken after ~2 hours at 5260 m whereas the ALT1 EPO sample was taken after ~10 hours at 5260 m. Samples were drawn into 10 mL syringes and immediately transferred into serum collection vacutainers (BD, Franklin Lakes, NJ, USA). These vacutainers were inverted 5 times and then allowed to sit for 30–60 minutes at room temperature to allow for proper clotting. Tubes were spun for 20 minutes at 800 relative centrifugal force at room temperature. Once separated, serum was stored on ice for 10 minutes before being stored in either a −80°C freezer (Eugene), or in a charged nitrogen vapor shipper (Bolivia). Frozen serum samples were transported in charged nitrogen vapor shippers, and then stored at −80°C until analysis. Serum ferritin was assessed via nephelometry (within-run coefficient of variation (CV): 7%; between-run CV: 5%; Siemens BNII Nephelometer, Erlangen, Germany). Serum EPO was assessed in duplicate using a Quantikine IVD Human Epo ELISA kit (intra-assay CV: 4%; inter-assay CV: 6%; R&D Systems, Minneapolis, MN, USA).

Missing Data. As mentioned above, 6 Hbmass tests were excluded due to CO leaks. We also missed Hbmass tests due to logistical difficulties (6 tests) and subject discomfort prior to Hbmass testing (1 test). Some v[Hb] or vHct samples (total of 16 tests) were missed for logistical reasons and difficulties with obtaining or processing venous samples; in the case of missing v[Hb] or vHct, data were also excluded from the analysis of changes in BV parameters. The majority of missing tests for Hbmass and BV occurred at ALT1. Ferritin values were missing from 3 tests and EPO values were missing from 8 tests.

Statistics. Statistical analyses were performed using Statistical Package for the Social Sciences (version 20, SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2008 (Redmond, WA, USA). We performed linear mixed model statistical analyses to examine our outcome variables across acclimatization using the Mixed procedure in SPSS. A major advantage of linear mixed model statistical analyses is that missing values do not result in casewise deletion of other longitudinal measurements, as is required with repeated-measures analysis of variance. Time (SL, ALT1, ALT7, ALT16), sex, and a time × sex interaction were included in the linear mixed models as fixed factors. Time comparisons were made with SL as the reference. Separate paired-tests were performed to compare ALT7 and ALT16. No adjustments were made for multiple testing. Due to largely reduced subject numbers at ALT1 for Hbmass/BV parameters, we could not be certain that the missing data at ALT1 met the missing-at-random requirement of the linear mixed model. Therefore, we performed paired t-tests to examine changes between SL and ALT1. To examine differences at POST7/POST21 compared to SL and ALT16, we used linear mixed models with time (SL, ALT16, POST), group (POST7, POST21), and a time × group interaction included as fixed factors. The POST timepoint includes POST7 and POST21 measurements,

with differences between the groups descending for 7 versus 21 days assessed by comparing the effect of group. Time comparisons were made with POST as the reference–therefore, data from SL and ALT16 were excluded from these analyses for subjects missing the POST7/POST21 time point. Due to the reduced number of female subjects included at POST21 (n = 2), sex was not included in these models. We performed simple linear regressions to examine relationships between variables. For all analyses, statistical significance was accepted when $p \leq 0.05$. Data are presented throughout the paper as mean ± SD unless otherwise noted. A complete list of individual data for Hbmass and serum ferritin is provided in Table S1.

Results

Subject characteristics and ferritin status during high altitude acclimatization and de-acclimatization

A detailed description of subject characteristics is presented elsewhere [22] –briefly, body mass was reduced by 2.6±1.6 kg after 16 days of high altitude exposure. Serum ferritin levels are presented in Table 1. Ferritin levels were lower in women compared to men. At ALT1, all men had ferritin above 20 ng mL^{-1}, whereas 4 of 8 women had ferritin levels below this value. Ferritin levels decreased from ALT1 to ALT16 in both men (−68±16%) and women (−65±26%) and increased following descent from high altitude in both men (+189±196%) and women (+184±283%).

Hematological adaptations during 16 days of high altitude acclimatization

EPO increased from a baseline level of 8.3±5.0 IU L^{-1} by 4.9±2.8 fold at ALT1 (n = 16), 8.3±8.7 fold at ALT7 (n = 18), and 2.5±1.6 fold at ALT16 (n = 21; all $p<0.05$ compared to SL). There were no significant correlations between the increases in EPO at ALT1, ALT7, or ALT16 and changes in Hbmass. Data comparing SL and ALT1 for subjects with Hbmass/BV measurements at both time points are presented separately in Table 2 so that the effect of acute altitude on Hbmass and BV parameters can be distinguished from inter-individual variation. We found a non-significant 11 g loss in absolute Hbmass at ALT1 compared to SL (p = 0.206); there was also a trend for relative Hbmass to be slightly (0.3 g kg^{-1}) reduced at ALT1 compared to SL (p = 0.056). A small decrease in Hbmass was expected, as Hbmass was assessed after the required blood sampling for other protocol procedures on ALT1. v[Hb], vHct, and BV compartments were not significantly different at ALT1 compared to SL.

Table 3 presents data on hematological parameters at SL, ALT7, and ALT16. Compared to SL, absolute Hbmass was increased at ALT7 (+3.7±5.8%; n = 20; $p<0.01$) and ALT16 (+7.6±6.6%; n = 21; $p<0.001$), with the gain larger at ALT16 compared to ALT7. The increase in absolute Hbmass was larger in men compared to women at ALT16. Relative Hbmass was increased compared to SL at ALT7 and ALT16; relative Hbmass was greater at ALT16 compared to ALT7 and the increases were greater in men than women at both ALT7 and ALT16. Due to the lower absolute and relative Hbmass levels in women compared to men, we also examined the percent change in absolute Hbmass from SL and found no significant difference between men and women (Figure 1A). At ALT16, Hbmass was elevated compared to SL in all 12 men and 7 out of 9 women. There was no significant correlation between ferritin level upon initial exposure to altitude and the percent change in absolute Hbmass at ALT16 (Figure 1B).

Table 1. Serum ferritin during high altitude acclimatization and de-acclimatization.

		Time				Significant Effects	
		SL	ALT1	ALT16	POST	Sex	Time
Serum Ferritin (ng ml^{-1})						M>W	ALT16 <SL; ALT16<ALT1; ALT16<POST
	M	63.2±29.0 (12)	66.8±42.2 (11)	25.8±24.0 (12)	52.3±44.0 (11)		
	W	28.9±15.5 (9)	19.7±10.9 (8)	7.5±5.2 (9)	19.6±21.3 (9)		

Data are presented as mean ± SD (ng ml^{-1}) with the number of subjects indicated in parentheses. The POST measurement took place upon initial return to 5260 m following descent to 1525 m for 7 days (7W, 7M) or 21 days (2W, 4M) duration. Linear mixed model statistical analyses were performed to examine the effects of sex and time with SL as the reference. Paired t-tests were performed to compare ALT16 with ALT1 and POST. Effects were accepted as significant when $p \leq 0.05$.

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Table 2. Hematological parameters at sea level and the first day of exposure to 5260 m.

	SL	ALT1	Percent Change
Hbmass (g)	723±175 (6,6)	711±173 (6,6)	−1.6±4.4%
Rel Hbmass (g kg^{-1})	10.1±1.4 (6,6)	9.8±1.4 (6,6)	−2.7±4.4%
v[Hb] (g dL^{-1})	13.9±1.0 (8,4)	14.3±1.5 (8,4)	2.5±6.7%
vHct (%)	42.6±2.6 (8,4)	42.6±4.5 (8,4)	−0.3±6.5%
BV (ml)	6224±848 (5,3)	6130±517 (5,3)	−0.8±7.5%
Rel BV (ml kg^{-1})	83.2±6.6 (5,3)	81.9±7.3 (5,3)	−1.4±7.0%
PV (ml)	3907±416 (5,3)	3866±276 (5,3)	−0.2±11.5%
Rel PV (ml kg^{-1})	52.4±4.7 (5,3)	51.9±7.3 (5,3)	−0.9±10.9%
RCV (ml)	2317±442 (5,3)	2264±417 (5,3)	−2.1±5.0%
Rel RCV (ml kg^{-1})	30.8±3.0 (5,3)	29.9±3.3 (5,3)	−2.6±5.6%

Data are presented as mean ± SD with the number of subjects (M,W) indicated in parentheses. This table only includes data for subjects with measures at both time points so that the effect of acute altitude on Hbmass and BV parameters can be distinguished from the inter-individual variation. Paired t-tests were performed for each parameter and none of the differences were statistically significant (all $p>0.05$).
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v[Hb] and vHct were increased at ALT7 and ALT16 compared to SL, with no significant differences between ALT7 and ALT16. Men had larger increases in v[Hb] and vHct at ALT16 compared to women. Absolute and relative PV were reduced at ALT7 and ALT16 compared to SL, with no significant difference between ALT7 and ALT16. The reduction in absolute PV at ALT16 was greater in men compared to women, but no significant difference was detected in the change in relative PV. Absolute BV was reduced at ALT7 and ALT16 compared to SL, with no significant difference between ALT7 and ALT16 or in the change in BV between men and women. Relative BV was not significantly different from SL at ALT7 or ALT16, but there was a trend ($p = 0.057$) for women to have a greater reduction in relative BV compared to men at ALT7. Relative BV was greater at ALT16 compared to ALT7. Changes in absolute and relative RCV mirrored changes in Hbmass.

Hematological adaptations following descent to low altitude

Table 4 presents hematological parameters for subjects with complete measurements at SL, ALT16, and POST7/POST21. For all hematological parameters, there were no significant differences in responses between POST7 and POST21 groups or any significant group × time interactions. Absolute (−6.0±3.7%) and relative (−6.8±4.3%) Hbmass declined following high altitude descent–absolute Hbmass at the POST7/POST21 measurement was not significantly different from SL (+0.8±4.5%), but relative Hbmass was slightly elevated compared to SL (+3.2±5.5%). Figure 2A shows the percent changes in absolute Hbmass from SL at ALT16 and POST7/POST21. A similar pattern was observed for RCV, with absolute and relative RCV values reduced at POST7/POST21 compared to ALT16. Absolute and relative PV were increased at POST7/POST21 compared to ALT16 and not significantly different from SL. Absolute and relative BV at POST7/POST21 were not significantly different from ALT16 or SL. v[Hb] and vHct were reduced at POST7/POST21 compared to ALT16 and not significantly different compared to SL.

The gain in Hbmass from SL to ALT16 was correlated with the reduction in Hbmass from ALT16 to POST7/POST21 (Figure 2B; $r = -0.77$; $n = 20$; $p = 0.00006$). The reduction in Hbmass from ALT16 to POST7 was correlated with an increase in serum ferritin (Figure 2C; $r = -0.64$; $n = 13$; $p = 0.02$).

Discussion

This study provides the first data on early Hbmass alterations in healthy humans with ascent to and descent from altitudes greater than 5000 m. We found an increase in Hbmass at ALT7 and a further augmentation by ALT16. However, the altitude-induced gain in Hbmass was remarkably short-lived, as descent to low altitude resulted in a reduction in Hbmass to baseline values within 7 days. The correlation between the loss in Hbmass and increase in serum ferritin following descent to low altitude suggests that this rapid reduction in Hbmass was mediated by increased red blood cell destruction. Overall, this study demonstrates the capacity for rapid alterations in Hbmass with ascent to and descent from high altitude and suggests the need to further examine mechanisms of erythropoietic adaptations to severe hypoxia.

Increase in Hbmass during high altitude acclimatization

The veracity of our finding of swift alterations in Hbmass is predicated on the validity and sensitivity of our methodological approach for measuring Hbmass. We have several reasons to believe our measurements were robust and that our findings were not the result of analytical error or artifact. First, CO rebreathing methods have been shown to have low measurement error compared to other methodological approaches for assessing the red cell compartment [39] and we achieved a measurement error of 1.5% from duplicate baseline tests in the present study. At ALT7 and ALT16, the mean increases in Hbmass we observed were 2–5 times greater than our measurement error. Second, we performed quality-control analysis for ΔHbCO% both at SL and high altitude and found near-identical results [34]. Third, the ALT7 and ALT16 measures, at which Hbmass was elevated compared to SL, took place at the same location and with the same equipment and personnel as the POST7 and POST21 measures, at which Hbmass had returned to SL values following a 7 or 21 day de-acclimatization period. Therefore, a spurious inflation of Hbmass only at ALT7 and ALT16 seems unlikely. Taken together, we are confident that our findings reflect true physiological alterations in Hbmass with early ascent to and

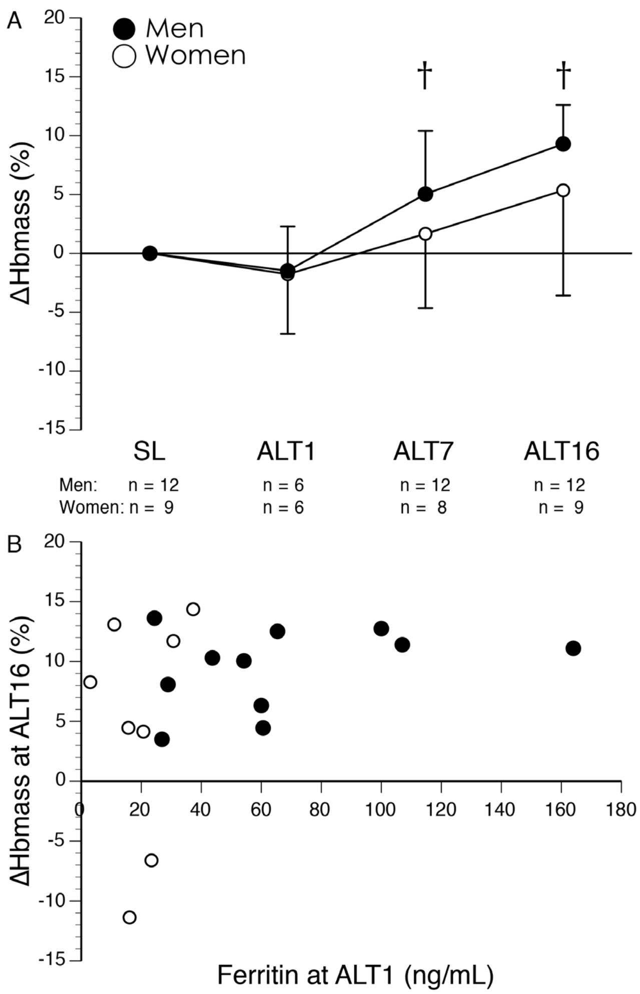

Figure 1. Hemoglobin mass in men and women during 16 days high altitude acclimatization. A) Time course of changes in absolute Hbmass. Data are presented as mean ± SD, with the number of men and women tested at each time indicated below the x-axis. †Significantly different from sea level ($p<0.05$; main effect of time). The percent changes were not significantly different between men and women ($p>0.05$). B) Relationship between serum ferritin level upon arrival at high altitude and the percent change in absolute Hbmass following 16 days at high altitude. Two subjects had missing ferritin data at ALT1 and their Hbmass data were excluded from this graph. There was no correlation between initial ferritin level upon arrival at altitude and the percent change in absolute Hbmass during high altitude acclimatization ($r = 0.33$; $n = 19$; $p = 0.16$).
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descent from high altitude and were not caused by normal biological variation or analytical error.

The speed of change in Hbmass observed in our study conflicts with the classical dogma that increases in Hbmass during high altitude acclimatization take at least 3–4 weeks to develop [2,3]. Recent studies at moderate altitude (2000–3600 m) suggested that erythropoietic adaptations may be swifter than previously thought [13,14,15,17], but the speed and magnitude of increase in Hbmass we observed at ALT7 and ALT16 exceed previous findings. The magnitude of change we observed is particularly striking given that some Hbmass was lost due to sampling (estimated loss of 29±12 g at ALT1, 10±4 g at ALT7, and 30±4 g at ALT16). We report the Hbmass values that we measured without adjusting for the blood lost due to sampling–this underestimates the total increase in the amount of hemoglobin produced at high altitude (estimate presented in Figure 3), which includes the Hbmass change we measured above our SL baseline values plus the Hbmass lost due to blood sampling on ALT1, ALT7 and ALT16 combined (calculated as 69±16 g; $n = 21$). Our findings raise the intriguing question of what mechanism enables this rapid and robust increase in Hbmass in response to severe hypoxia.

The regulation of red cell production is known to be largely influenced by EPO [42]. EPO peaks within the first 2–3 days of altitude exposure before beginning to fall towards baseline [40,41] and it has been suggested that the rapid return of EPO to baseline levels with continued altitude exposure should reduce the magnitude of the erythropoeitic stimulus compared to exogenous EPO treatment [2]. However, single measurements of circulating EPO do not adequately reflect the complex kinetics of EPO secretion over days and weeks, and it is unclear if the fall in circulating EPO with continued altitude exposure results from a decrease in EPO expression or is related to an increased rate of clearance from circulation [42]. It is noteworthy that much of the RCV expansion in lowlanders ascending to 4540 m for a 12-month period occurred after 1–2 months [7], a time at which EPO would be expected to have returned to baseline [40,41]. Importantly, our finding of an increase in Hbmass within 7 days of ascent to high altitude is in stark contrast to studies involving exogenous EPO administration, where Hbmass has been consistently reported to remain unchanged within the first 12 days of treatment [4,5,6] despite continuous elevation of circulating EPO above baseline [4]. In comparing high altitude ascent with EPO treatment, it is important to consider that the distinct stimuli of high altitude residence *versus* pharmacological EPO administration are markedly different. Whereas the elevation in EPO with severe hypoxia is secondary to hypoxia-inducible factor (HIF) signaling [43], the provision of exogenous EPO bypasses broad HIF activation. Differences between these conditions are reflected in divergent responses in other pathways affected by HIF signaling that influence erythopoietic adaptations such as iron mobilization [4,43,44]. Ultimately, we cannot provide mechanistic data from our study explaining the swifter increase in Hbmass in our subjects compared to previous studies at lower elevations or involving exogenous EPO administration, and we are not suggesting that EPO is not a key player in augmenting erythropoiesis in response to severe hypoxia. Rather, the more rapid increase in Hbmass in severe hypoxia compared to exogenous EPO treatment suggests that mechanisms in addition to augmentation of EPO may play an important role in the rapid erythropoietic response.

To our knowledge, we are the first to compare changes in Hbmass/RCV in men and women at identical time points and under similar experimental conditions at altitudes greater than 4000 m. We found that the percent increase in absolute Hbmass following 16 days at high altitude was not significantly different between men and women. Some previous cross-sectional studies of moderate altitude residents have suggested that erythropoietic responses may be lower in females compared to males [45,46], and it has been suggested that the ventilatory-stimulating effects of the female sex hormones play a key role [47]. However, Reeves et al. found no effect of menstrual phase on ventilatory or erythropoietic adaptations in healthy women acclimatizing to 4300 m despite large differences in sex hormone levels between subjects in the luteal versus follicular phases [12]. Arterial oxygen pressure and saturation did not differ between men and women at ALT1 and therefore the impact of ventilatory effects on potential sex differences in erythropoietic responses to high altitude would be minimal in our study.

Table 3. Hematological adaptations during 16 days high altitude acclimatization in healthy men and women.

		Time			Significant Effects		
		SL	ALT7	ALT16	Sex	Time	Interaction
Hbmass (g)					**M>W**	**ALT7>SL; ALT16>SL; ALT16>ALT7**	**ΔM>ΔW ALT16**
	M	905±95 (12)	950±110 (12)	989±110 (12)			
	W	559±62 (9)	567±76 (8)	590±91 (9)			
Rel Hbmass (g kg^{-1})					**M>W**	**ALT7>SL; ALT16>SL; ALT16>ALT7**	**ΔM>ΔW ALT7; ΔM>ΔW ALT16**
	M	11.9±1.4 (12)	13.0±1.7 (12)	13.8±1.6 (12)			
	W	9.1±0.8 (9)	9.2±1.0 (8)	9.7±1.2 (9)			
v[Hb] (g dL^{-1})					**M>W**	**ALT7>SL; ALT16>SL**	**ΔM>ΔW ALT16**
	M	14.6±0.6 (12)	16.2±1.1 (12)	16.9±0.6 (12)			
	W	12.4±0.8 (9)	13.7±1.1 (8)	13.4±1.0 (8)			
vHct (%)					**M>W**	**ALT7>SL; ALT16>SL**	**ΔM>ΔW ALT16**
	M	44.2±1.4 (12)	48.5±3.0 (12)	50.6±2.2 (12)			
	W	39.1±2.3 (9)	42.4±2.8 (8)	42.1±3.3 (8)			
BV (ml)					**M>W**	**ALT7<SL; ALT16<SL**	
	M	6813±667 (12)	6434±687 (12)	6420±588 (12)			
	W	4974±565 (9)	4592±730 (7)	4821±756 (8)			
Rel BV (ml kg^{-1})					**M>W**	**ALT16>ALT7**	
	M	89.9±9.3 (12)	88.0±8.9 (12)	89.5±8.0 (12)			
	W	80.9±7.1 (9)	74.2±7.8 (7)	78.7±9.8 (8)			
PV (ml)					**M>W**	**ALT7<SL; ALT16<SL**	**ΔM>ΔW ALT16**
	M	4190±412 (12)	3713±473 (12)	3580±304 (12)			
	W	3287±417 (9)	2921±502 (7)	3047±499 (8)			
Rel PV (ml kg^{-1})						**ALT7<SL; ALT16<SL**	
	M	55.3±5.5 (12)	50.7±5.5 (12)	49.9±3.8 (12)			
	W	53.4±5.6 (9)	47.2±5.5 (7)	49.8±6.9 (8)			
RCV (ml)					**M>W**	**ALT7>SL; ALT16>SL; ALT16>ALT7**	
	M	2623±279 (12)	2721±298 (12)	2839±331 (12)			
	W	1687±188 (9)	1671±247 (7)	1773±300 (8)			
Rel RCV (ml kg^{-1})					**M>W**	**ALT7>SL; ALT16>SL; ALT16>ALT7**	**ΔM>ΔW ALT7; ΔM>ΔW ALT16**
	M	34.6±4.0 (12)	37.3±4.6 (12)	39.6±4.8 (12)			
	W	27.4±2.3 (9)	27.1±2.8 (7)	28.9±4.0 (8)			

Data are presented as mean ± SD with the number of subjects indicated in parentheses. Linear mixed model statistical analyses were performed to examine the effects of sex, time (with SL as the reference) and a sex × time interaction. Paired t-tests were performed to compare ALT7 with ALT16. Effects were accepted as significant when $p \leq 0.05$.

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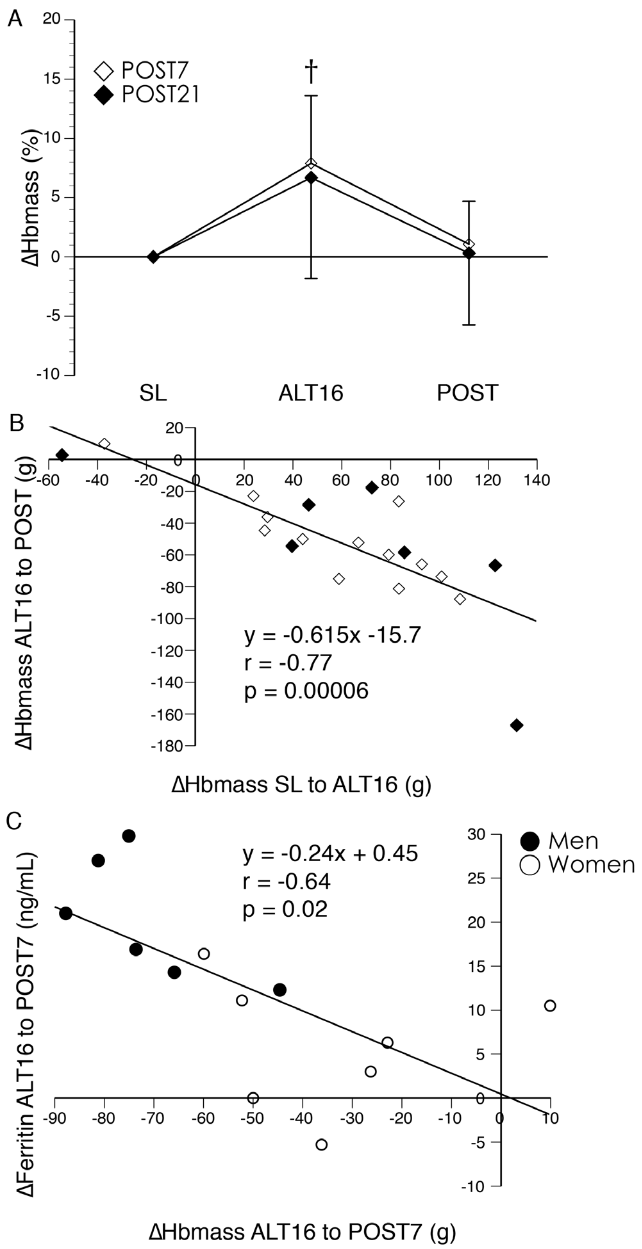

Figure 2. Change in hemoglobin mass following descent from high altitude to low altitude. Subjects were tested at high altitude at the end of a 16 day acclimatization period and upon return to high altitude after descent to low altitude (1525 m) for either 7 (POST7; n = 13) or 21 days (POST21; n = 7). Data are presented as mean ± SD. A) Changes in Hbmass. † Significantly different from POST ($p<0.05$; main effect of time). There were no significant differences between the POST7 and POST21 groups or between POST and SL ($p>0.05$). B) Relationship between changes in Hbmass following 16 days high altitude acclimatization and changes in Hbmass following descent to low altitude. C) Relationship between changes in Hbmass and changes in serum ferritin following descent to low altitude for 7 days.
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Our finding that the percent change in absolute Hbmass did not differ between men and women is particularly striking given the low ferritin levels of our female subjects upon arrival at high altitude. Although subjects with low ferritin during baseline testing were directed to take oral iron supplements up until departure for high altitude, several women arrived at high altitude with low ferritin levels, and based on previous work at moderate altitude [48], one might expect that the low iron stores would prevent an increase in Hbmass. In contrast, most (7 out of 9) of the women increased Hbmass in response to high altitude exposure. However, while all 12 men had increases in Hbmass following 16 days high altitude acclimatization, 2 women failed to increase Hbmass; indeed, these 2 women had reductions in Hbmass that were similar to the calculated amount of Hbmass withdrawn from these subjects for blood sampling at altitude. We examined the EPO response of these 2 individual subjects and found that their increases in EPO with high altitude exposure were above the group median at ALT1, ALT7, and ALT16, suggesting that the failure to increase Hbmass was not caused by a lack of EPO upregulation. As can be observed in Figure 1B, these 2 women were not distinguished by particularly low iron stores upon arrival at high altitude. Indeed, our individual data demonstrate the capability to increase Hbmass despite low ferritin levels upon initial arrival at high altitude. The subject with the lowest ferritin (3 ng mL^{-1}) upon initial high altitude exposure had a relatively large (8.3%) increase in Hbmass.

It might be questioned whether the level of storage iron indicated by these low ferritin values would be sufficient to enable a large increase in Hbmass. However, previous work has demonstrated increases in intestinal iron absorption at high altitude [7,49,50] and it is possible that dietary iron intake (not measured in the current study) provided sufficient iron for increasing hemoglobin production. Additionally, recent data suggest that a decrease in skeletal muscle iron content during the first week at high altitude may increase iron available for erythropoiesis [44]. Admittedly, because we did not measure dietary iron intake or iron-related proteins in skeletal muscle, the role of these mechanisms in allowing increased erythropoiesis in our subjects with low ferritin is purely speculative. However, these other studies highlight the complexity of iron homeostasis at high altitude and suggest that the iron required for increasing erythropoiesis may have been obtained from increased intestinal absorption or mobilization of iron from skeletal muscle stores. Further studies involving Hbmass assessments coupled with more comprehensive assessments of iron homeostasis are needed to more robustly determine the relationship between iron availability and erythropoiesis at high altitude. Our data suggest that low initial iron stores do not requisitely prevent high altitude-induced erythropoiesis; however, we stress that we cannot determine from our data whether low iron stores limited the magnitude of increase in Hbmass in some subjects.

Decrease in Hbmass following descent from high altitude to low altitude

We found that the Hbmass gained during high altitude acclimatization was quickly lost following descent to 1525 m. Hbmass had returned to SL baseline in our subjects who descended to low altitude for 7 days, and there was no further decrement in the group who descended for 21 days. To our knowledge, we are the first to report a complete loss of altitude-induced Hbmass adaptation within 7 days; the speed of this de-acclimatization response contrasts with previous studies, in which Hbmass has been reported to remain elevated above baseline for multiple weeks following descent to SL [14,15,17,51]. Although a rapid loss in RCV following high altitude descent has been previously reported by Rice et al. [21], there are several aspects of our study that make our findings unique. We studied lowlanders following just 16 days of high altitude acclimatization whereas Rice et al. [21] studied polycythemic high altitude natives. This was reflected by dramatic differences in the degree of polycythe-

Table 4. Hematological adaptations following descent from high altitude to low altitude.

	Time			Significant Effects
	SL	**ALT16**	**POST**	**Time**
Hbmass (g)				**POST<ALT16**
POST7	726±172 (6,7)	785±194 (6,7)	734±173 (6,7)	
POST21	802±245 (5,2)	865±296 (5,2)	810±266 (5,2)	
Rel Hbmass (g kg^{-1})				**POST<ALT16; POST>SL**
POST7	10.2±1.4 (6,7)	11.3±1.9 (6,7)	10.5±1.6 (6,7)	
POST21	11.7±2.4 (5,2)	13.3±3.3 (5,2)	12.3±2.9 (5,2)	
v[Hb] (g dL^{-1})				**POST<ALT16**
POST7	13.6±1.2 (5,4)	15.4±1.6 (5,4)	13.8±1.5 (5,4)	
POST21	14.2±1.1 (5,2)	16.0±2.1 (5,2)	14.7±1.8 (5,2)	
vHct (%)				**POST<ALT16**
POST7	42.3±2.9 (5,4)	47.0±4.3 (5,4)	42.0±4.1 (5,4)	
POST21	43.1±2.9 (5,2)	48.5±6.1 (5,2)	44.4±4.1 (5,2)	
BV (ml)				
POST7	6017±907 (4,4)	5694±926 (4,4)	5965±825 (4,4)	
POST21	6125±1569 (5,2)	5820±1476 (5,2)	5914±1445 (5,2)	
Rel BV (ml kg^{-1})				
POST7	83.6±5.2 (4,4)	81.4±7.1 (4,4)	85.0±6.0 (4,4)	
POST21	90.1±13.2 (5,2)	90.2±12.5 (5,2)	90.9±12.0 (5,2)	
PV (ml)				**POST>ALT16**
POST7	3813±435 (4,4)	3363±403 (4,4)	3775±423 (4,4)	
POST21	3799±885 (5,2)	3300±664 (5,2)	3587±749 (5,2)	
Rel PV (ml kg^{-1})				**POST>ALT16**
POST7	53.2±3.1 (4,4)	48.3±3.0 (4,4)	54.0±4.4 (4,4)	
POST21	56.1±6.9 (5,2)	51.5±4.0 (5,2)	55.4±5.3 (5,2)	
RCV (ml)				**POST<ALT16**
POST7	2204±485 (4,4)	2331±556 (4,4)	2190±480 (4,4)	
POST21	2326±695 (5,2)	2520±847 (5,2)	2326±711 (5,2)	
Rel RCV (ml kg^{-1})				**POST<ALT16**
POST7	30.4±3.6 (4,4)	33.2±5.3 (4,4)	31.0±4.2 (4,4)	
POST21	34.0±6.7 (5,2)	38.7±9.4 (5,2)	35.5±7.3 (5,2)	

Data are presented as mean ± SD with the number of subjects (M,W) indicated in parentheses. Subjects were studied at sea level, at 5260 m after 16 days high altitude acclimatization, and upon initial return to 5260 m after descent to 1525 m for 7 (POST7) or 21 (POST21) days. Linear mixed model statistical analyses were performed to examine the effects of time (with POST as the reference), group (POST7 versus POST21) and a time × group interaction. Effects were accepted as significant when $p \leq 0.05$. There were no significant effects of group or any significant group × time interactions (all $p > 0.05$).

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mia obtained at high altitude (mean [Hb] of 23.4 g dL^{-1} in the high altitude natives versus 15.5 g dL^{-1} at ALT16 in our subjects). Indeed, the majority of subjects in the study of Rice et al. [21] met the criteria for excessive erythrocytosis ([Hb]≥21 in men or ≥19 in women; [52]) whereas none of our subjects came within 2 g dL^{-1} of this criterion at ALT16. Therefore, our results show that the development of excessive polycythemia is not required for high altitude descent to induce a rapid loss in Hbmass.

Based on the kinetics of red blood cell turnover (~0.83% of circulating cells destroyed per day [53]) and the delayed influence of changes in EPO on red blood cell production [21,42], the large reduction in Hbmass we observed within 7 days is unlikely to be explained by a reduction in red blood cell production. The correlation between the loss in Hbmass and increase in serum ferritin from ALT16 to POST7 suggests an increase in red blood cell destruction, as the iron contained in destroyed red blood cells is transferred to iron stores [21]. It is possible that neocytolysis, the selective destruction of a population of young red blood cells [54,55,56], may have been the mechanism of this rapid loss in Hbmass. However, the strength of the evidence for neocytolysis has recently been questioned [57]. We did not measure markers of red blood cell production or examine red blood cell age distributions during high altitude acclimatization and de-acclimatization and therefore cannot provide direct evidence in support of, or against a role for, neocytolysis.

Our finding of a rapid loss in Hbmass following descent from high altitude contrasts with patterns observed following cessation of exogenous EPO administration [5,6,20], despite the fact that these studies induced similar or larger elevations in Hbmass and [Hb]/Hct compared to our observations. Although it was hypothesized that cessation of exogenous EPO therapy would induce neocytolysis and lead to a rapid reduction in Hbmass

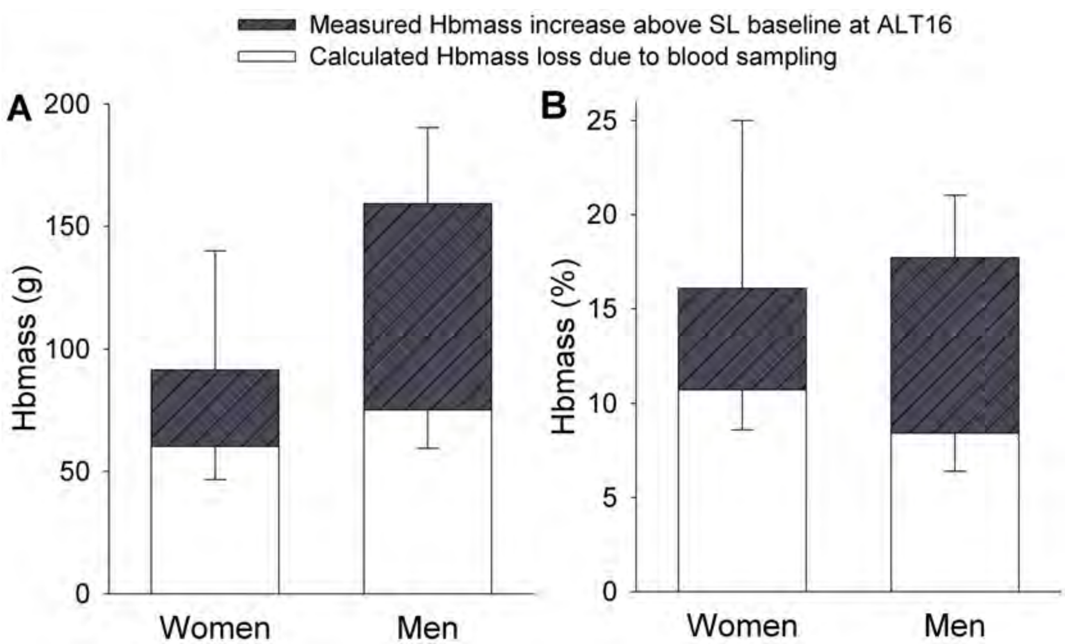

Figure 3. Estimate of the increase in hemoglobin mass produced during 16 days high altitude acclimatization determined from the measured Hbmass increase above sea level baseline plus the calculated Hbmass loss due to blood sampling. Panel A represents the absolute increase in Hbmass (g) produced. Panel B represents the percent increase in Hbmass produced. Data are presented as mean ± SD. Upward SD bars represent the SD of the increase in Hbmass measured above baseline and the downward SD bars represent the SD of calculated Hbmass loss due to blood sampling.
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[21,56], recent studies provide compelling evidence that this is not the case, with Hbmass/RCV consistently reported to remain unchanged for 2 weeks following treatment cessation before beginning to fall gradually back to baseline [5,6,20]. The stimulus for increased red blood cell production with high altitude acclimatization is hypobaric hypoxia, whereas EPO treatment elevates Hbmass in the absence of systemic hypoxia. Although this suggests that the production of red blood cells under conditions of hypoxia may influence the retention of Hbmass adaptations, it is important to note that rapid reductions in Hbmass have also been reported with spaceflight [54] and dehydration-induced rapid weight loss [58] and neither of these situations involve systemic hypoxia. Further work is required to clearly establish the mechanism(s) of rapid loss of Hbmass in healthy humans.

What are the implications of the rapid loss in Hbmass following descent to low altitude on acclimatization status upon return to high altitude? The reduction in oxygen carrying capacity might be expected to impair submaximal endurance performance upon return to 5260 m; however, despite large reductions in absolute and relative Hbmass from ALT16 to POST7, the improvement in 3.2 km run time-trial performance from ALT1 to ALT16 with acclimatization was fully maintained at POST7 [22]. This calls into question the importance of the altitude-induced Hbmass adaptation for submaximal endurance performance at high altitude. Previous studies examining the effects of artificial Hbmass alterations with erythrocyte infusion [59,60], recombinant EPO treatment [61], and isovolemic hemodilution [62] have failed to observe alterations in maximal oxygen uptake or endurance performance at altitudes greater than 4300 m. Our results extend these findings by showing that the loss in Hbmass accompanying descent to low altitude does not result in impaired submaximal endurance performance upon return to high altitude. However, previous work suggests that there may be a threshold altitude above which alterations in Hbmass have minimal effects on maximal oxygen uptake [61], and we stress that our finding of maintained endurance performance despite significant loss of Hbmass may not apply to performances at less severe altitudes.

Changes in blood volume compartments during high altitude acclimatization and de-acclimatization

In support of previous high altitude studies (Reviewed in [2]), we observed large reductions in absolute and relative PV at ALT7 and ALT16. However, we did not detect a significant reduction in PV in the first 9–13 hours of initial exposure to 5260 m and the PV measured 9–13 hours after return to 5260 m following descent to low altitude was not different from SL. Some studies have found reductions in PV within the first hours of high altitude exposure [9,10] but other studies have failed to detect changes within this time window [23,24]. Differences between studies are likely influenced by several factors including hydration status, exercise prior to PV assessments, acute mountain sickness, and the methodology used to assess PV. PV returned to SL values following descent to low altitude. Previous work has indicated that the recovery of PV following high altitude descent occurs within 2 days and is influenced by changes in fluid-regulating hormones including renin, aldosterone, and vasopressin [26,27], so our finding of a recovered PV following 1 and 3 weeks at low altitude is not surprising. As expected, changes in RCV paralleled the changes we observed in Hbmass. Compared to SL, relative BV was unchanged by high altitude acclimatization and de-acclimatization, at least at the time points we assessed–relative BV was influenced both by alterations in the absolute sizes of the BV compartments and small changes in body mass [22] during high altitude acclimatization and de-acclimatization. In our subjects, reductions in relative PV during high altitude acclimatization were offset by an augmentation of relative RCV. The opposite response

occurred following descent to low altitude, with the diminution in relative RCV offset by an enlargement of relative PV.

Limitations

There are some potential limitations to the current study that should be considered. The overall experimental design lacked a separate lowlander control group that was studied over time in the absence of altitude exposure. However, Hbmass has been consistently shown to be stable over time in subjects at SL [36,37] and we took several steps to ensure that the SL and high altitude measurements were comparable, as detailed above. Additionally, because examining responses to acute hypoxia on ALT1 was a key component of the overall AltitudeOmics study design, many steps were taken to minimize the subjects' exposure to hypoxia prior to ALT1 [22]. During the travel period prior to ALT1 (including flight time), subjects spent less than 20 hours exposed to hypoxia equivalent to 2000 m or greater. A recent meta-analysis of changes in Hbmass with hypoxia reported gains in Hbmass of ~1% per 100 hours spent above 2000 m [17]. Therefore, the effect of hypoxic exposure prior to ALT1 on the Hbmass response is estimated as less than 0.2%, dramatically lower than the increases we observed at ALT7 and ALT16.

As described previously, subjects were unable to maintain their normal physical activity habits at high altitude and some detraining may have occurred during acclimatization, with some fitness restoration during the period spent at low altitude [22]. Eastwood et al. found a 3.1% reduction in Hbmass after 30 days of detraining (~90% reduction in training volume) in triathletes at SL, but reported unchanged Hbmass at 10 and 20 days following training reduction [63]. A potential interaction between hypoxia and detraining on changes in Hbmass with ascent to high altitude has not been previously examined. There is a very strong cross-sectional relationship between lean body mass and Hbmass at sea level [64] and it could be speculated that the mean loss of ~1.5 kg lean body mass between ALT1 and ALT16 [22] may have reduced the erythropoietic stimulus. However, data examining a potential interaction between changes in lean body mass and Hbmass during altitude sojourn are currently lacking. Next, although subjects with low ferritin prior to baseline testing were directed to take oral iron supplements, supplementation was not directly monitored and the efficacy of supplementation in increasing serum ferritin was not determined prior to arrival at high altitude. Some subjects arrived at high altitude with low ferritin levels and it is possible that this may have limited the increase in Hbmass. However, as noted above, several subjects had robust increases in Hbmass despite low ferritin levels upon arrival.

Finally, the potential influence of blood loss due to sampling should be considered. Blood loss due to sampling occurs in many studies but its potential influence on hematological and other physiological outcomes is often ignored. The amount of blood removed due to sampling at ALT1 is of a magnitude that may induce a small EPO response at SL [65]. It is important to consider that it takes ~5 weeks to recover Hbmass lost from a 550 mL blood donation at SL [66], whereas our subjects were able to increase Hbmass above SL baseline within 7 days despite the loss of blood due to sampling. While we cannot rule out a potential interaction between blood loss due to sampling and the hypoxic stimulus on the magnitude of the erythropoietic response, it is clear that the hypoxic stimulus drives the rapid gain in Hbmass observed at 5260 m.

Conclusions

We documented the early time course of Hbmass adaptations at 5260 m and found rapid increases following just 7 and 16 days of high altitude acclimatization. The altitude-induced gain in Hbmass was remarkably short-lived, as descent to low altitude resulted in a dramatic loss in Hbmass within 7 days. The loss in Hbmass was correlated with an increase in serum ferritin, suggesting an increase in red blood cell destruction. Overall, this study demonstrates the capacity for rapid alterations in Hbmass with high altitude acclimatization and de-acclimatization in healthy men and women and suggests the need to further examine mechanisms of erythropoietic adaptations to severe hypoxia.

Supporting Information

Table S1 Individual hemoglobin mass data at SL, ALT1, ALT7, ALT16, POST7, and POST21 and serum ferritin data at SL, ALT1, ALT16, POST7, and POST21.
(PDF)

Acknowledgments

This paper is part of a series titled "AltitudeOmics" that together represent a group of studies that explored the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations invested enormous amounts of time and resources to make AltitudeOmics a success. Foremost, the study was made possible by the tireless support, generosity and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, Andrew T. Lovering, Andrew W. Subudhi and Robert C. Roach. A complete list of other investigators on this multinational, collaborative effort involved in development, subject management and data collection, supporting industry partners, and people and organizations in Bolivia that made AltitudeOmics possible is available elsewhere [22].

Author Contributions

Conceived and designed the experiments: BJR NBW WFS WCB CGJ ATL AWS RCR. Performed the experiments: BJR NBW. Analyzed the data: BJR NBW WFS WCB CGJ ATL AWS RCR. Wrote the paper: BJR NBW WFS WCB CGJ ATL AWS RCR.

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AltitudeOmics: Impaired pulmonary gas exchange efficiency and ventilatory acclimatization in humans with patent foramen ovale after 16 days at 5260 m

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Running title: Effect of PFO on acclimatization to high-altitude

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Abstract

A patent foramen ovale (PFO), present in ~40% of the general population, is a potential source of right-to-left shunt that can impair pulmonary gas exchange efficiency. However, prior studies investigating human acclimatization to high-altitude with pulmonary gas exchange as a key element have not investigated differences between subjects with (PFO+), or without a PFO (PFO−). We hypothesized that PFO+ subjects would have worse pulmonary gas exchange efficiency [increased alveolar-to-arterial PO_2 difference ($A\text{-}aDO_2$)] after acclimatization to high-altitude compared to PFO− subjects. Twenty-one (11 PFO+) healthy sea level residents were studied at rest and during cycle ergometer exercise at the highest iso-workload achieved at sea level (SL), after acute transport to 5260 m (ALT1), and after living at 5260 m for 16 days (ALT16). In contrast to the PFO− group, the PFO+ group had: 1) no improvement in $A\text{-}aDO_2$ at rest and during exercise at ALT16 compared to ALT1, 2) no significant increase in resting alveolar ventilation, alveolar PO_2, or arterial O_2 saturation at ALT16 compared to ALT1, and 3) a lower arterial PO_2 and higher arterial PCO_2 at rest at ALT16. These data suggest that right-to-left shunt through a PFO impairs pulmonary gas exchange efficiency even after acclimatization to high-altitude and PFO+ subjects may have reduced ventilatory acclimatization compared to PFO− subjects. In the presence of a PFO, it may be beneficial to have reduced ventilatory acclimatization that results in a right-shifted oxygen-hemoglobin dissociation curve to facilitate O_2 unloading at the tissue, offsetting the negative effects of PFO on O_2 loading.

Introduction

It is well established that pulmonary gas exchange progressively worsens in a workload dependent manner during exercise at sea level (10). This impairment in pulmonary gas exchange efficiency during exercise is exacerbated in acute hypoxia, such that for any given VO_2, the alveolar-to-arterial PO_2 difference (A-aDO_2) is greater compared to exercise in normoxia (62). Following acclimatization to hypobaric hypoxia, pulmonary gas exchange efficiency is thought to improve compared to acute hypoxia (22, 54). Seminal work from Dempsey *et al.* (9) reported a trend for an increased A-aDO_2 during treadmill walking after 4 days at 3100 m compared to sea level, and a partial normalization during the same exercise protocol following 21 days at 3100 m compared to that obtained after 4 days. Bebout *et al.* (4) then demonstrated that, compared to acute normobaric hypoxia, acclimatization to 3800 m for 2 weeks resulted in an ~3 mm Hg reduction in the A-aDO_2 during submaximal cycle ergometer exercise. Calbet *et al.* (8) subsequently reported that, compared to acute normobaric hypoxia, acclimatization to 5260 m for 9-10 weeks resulted in an ~9 mm Hg reduction in the A-aDO_2 during submaximal cycle ergometer exercise. Collectively, these data suggest that pulmonary gas exchange efficiency in non-acclimatized individuals improves with acclimatization to high-altitude compared to acute hypoxia.

Recently, our group has explored the consequences of an intracardiac right-to-left shunt via a patent foramen ovale (PFO) in healthy humans during exercise breathing room air and in acute hypoxia (37). In the course of these investigations it became apparent that the presence of a PFO could be critical to the interpretation of work where pulmonary gas exchange efficiency is a key element and, to our knowledge, prior work investigating human acclimatization to high-altitude have not considered the effect of a PFO. The classic study by Hagen *et al.* (19) reported a PFO prevalence of 25-35% identified using a probe during autopsy (*n* = 965). Recent work from several research groups using saline contrast echocardiography (*n* = 104-1162) report that ~40% of adult humans have a PFO (14, 40, 68). According to the multiplication rule for conditional probability and using a 35% prevalence of PFO, there is a <5%

chance that the aforementioned studies on pulmonary gas exchange efficiency after acclimatization would randomly select all subjects without PFO. Right-to-left blood flow through a PFO occurs when right atrial pressure exceeds left atrial pressure, which can occur transiently during normal respiration (16). Thus, right-to-left blood flow through a PFO is likely intermittent and variable in volume, however it does result in a measureable impact on pulmonary gas exchange efficiency. Lovering *et al.* (37) found that subjects with a PFO have an increased A-aDO_2 at rest, breathing either room air at sea level or a normobaric hypoxic gas mixture (12% O_2).

During conditions of elevated pulmonary pressures, right-to-left intracardiac shunt across a PFO could be exacerbated because of higher right atrial pressure exceeding left atrial pressure. Exaggerated pulmonary hypertension is a hallmark of high-altitude pulmonary edema (HAPE), a potentially life-threatening complication of sojourn to high-altitude, and the prevalence of PFO is >4 times higher in HAPE susceptible than HAPE resistant individuals (2). Moreover, systemic arterial oxygen desaturation, although unavoidable in acute hypobaric hypoxia, can be exacerbated by right-to-left intracardiac shunt, and is more pronounced in HAPE susceptible individuals (3). Taken together, arterial hypoxemia secondary to hypobaric hypoxia may be exacerbated in individuals with PFO.

Although individuals with PFO are suggested to be at an increased risk for the development of high-altitude illnesses such as HAPE (2) and AMS (34) very little is known regarding how the overall physiology of individuals with and without PFO differs at high-altitude. Indeed, prior studies investigating human acclimatization to high-altitude have not prospectively considered the effect of a PFO. Consequently, although pulmonary gas exchange efficiency and arterial hypoxemia are thought to improve following acclimatization to hypobaric hypoxia (4, 8, 9, 38) and high altitude illness incidence and severity decreases with acclimatization (18, 50), it remains unknown if these findings are generalizable to both individuals with and without PFO.

The primary purpose of this study was to investigate pulmonary gas exchange efficiency at rest and during exercise in subjects with and without a PFO after acclimatization to hypobaric hypoxia. We

hypothesized that in subjects with PFO, pulmonary gas exchange efficiency and arterial hypoxemia would not improve following acclimatization to high-altitude and that these subjects would be more susceptible to acute mountain sickness as a result of their greater arterial hypoxemia compared to PFO− subjects. To test this hypothesis, healthy male and female lowlanders, with and without a PFO, were studied at rest and during exercise at sea level, after being acutely transported to 5260 m, and after living at 5260 m on Mt. Chacaltaya, Bolivia, for 16 days. This study was conducted as part of the AltitudeOmics project, described previously in greater detail (57).

Methods

This study received approval from the University of Oregon, the University of Colorado Denver, and the U.S. Department of Defense. All subjects provided verbal and written informed consent prior to participation and all studies were conducted in accordance with the Declaration of Helsinki.

Subject recruitment and screening

A complete description of the inclusion/exclusion criteria were published in the project overview paper of this series (57). Briefly, 21 healthy subjects (9 female) recruited from sea level (Eugene, Oregon, 130 m, P_B = 749 mm Hg) participated in all aspects of this study and constitute the AltitudeOmics group of subjects in this report. Pertinent to the current report and not described in previous AltitudeOmics publications are the methodologies for determining adequate (≥90% predicted) pulmonary function and diffusion capacity for carbon monoxide parameters and the echocardiographic screening process each subject underwent.

Spirometry, diffusion capacity, and lung volumes

Baseline pulmonary function was determined using computerized spirometry (MedGraphics, Ultima CardiO_2, St. Paul, MN, USA) according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards (43). Lung diffusion capacity for carbon monoxide (DL_{CO}) was determined by the single-breath, breath hold method according to ATS/ERS standards (30, 39) using the Jones and Meade method for timing (26). Lung volumes and capacities were determined using whole body plethysmography (MedGraphics Elite Plethysmograph, St. Paul, MN, USA) according to ATS/ERS standards (64).

Echocardiographic screening

All subjects underwent a comprehensive echocardiographic screening process (Philips Sonos 5500, Eindhoven, The Netherlands) by a registered diagnostic cardiac sonographer with >25 yrs of experience (R.D.G.) to ensure subjects were free of cardiac abnormalities or signs of heart disease, as

previously conducted by our group (12–14, 32, 33, 45). Transthoracic saline contrast echocardiography (TTSCE) was used to identify the presence of a PFO as described previously (35). The appearance of ≥1 microbubble(s) in the left heart in any frame during the 20 cardiac cycles following right heart opacification identified subjects as either having a PFO or the transpulmonary passage of saline contrast (17, 41, 42, 51). Delineation between these two sources of left heart contrast results from the timing of contrast appearing in the left heart following right heart opacification, in which a microbubble appearing within ≤3 cardiac cycles is consistent with PFO, while a microbubble appearing after >3 cardiac cycles is consistent with transpulmonary passage (7, 24, 31, 41, 42, 51, 61). Saline contrast injections were performed during normal breathing as well as after provocative maneuvers designed to transiently elevate right atrial pressure and create conditions optimal for detection of PFO, specifically the release of a Valsalva maneuver. Effective Valsalva maneuvers, following a 15 sec strain phase, were confirmed by a transient leftward deviation of the interatrial septum upon release and multiple injections were performed as necessary when results were equivocal. PFO was defined by the appearance of ≥1 microbubble in the left heart within ≤3 cardiac cycles post right-heart opacification (7, 31). There were 11 (7 female) subjects with PFO (PFO+) and 10 (2 female) subjects without PFO (PFO−). Of note, we originally planned on having an equal number of PFO+ and PFO− subjects, although for reasons previously described 3 subjects were excluded (57).

Timeline

This report presents data collected over 3 experimental study visits: 1) sea level (SL: Eugene, Oregon, 130 m, P_B = 749 mm Hg); 2) day 1 at high-altitude (ALT1: Mt. Chacaltaya, Bolivia, 5260 m, P_B = 406 mm Hg); and 3) after living at high-altitude for 16 days (ALT16: Mt. Chacaltaya, Bolivia, 5260 m, P_B = 406 mm Hg). To provide an acute exposure to 5260 m on ALT1, the subjects breathed supplemental oxygen (2 L/min, nasal cannula or mask) during the drive up the mountain. On arrival at 5260 m, the first subject immediately began the experimental protocol while the second subject rested while

continuing to breathe supplemental oxygen for ~2 hr until the first subject had completed the protocol. A complete description of the study timeline was previously published (57).

Subject instrumentation and exercise protocol

A core temperature pill (CorTemp HQInc., Palmetto, FL, USA) was ingested ~5 hrs prior to the start of exercise. Subjects were instrumented with a 20 G radial artery catheter (Arrow International Inc., Reading, PA, USA) under local anesthesia (2% lidocaine) and an 18-22 G intravenous catheter was placed in the antecubital fossa. Subjects rested on an upright stationary cycle ergometer (Velotron Elite, Seattle, WA, USA) for 10 min prior to performing standardized workloads of 70, 100, 130, and 160 W for 3 min each. Exercise data are presented as the highest iso-workload achieved within an individual subject across SL, ALT1 and ALT16. For example, subjects may have completed all workloads at SL and ALT16, but only completed 130 W at ALT1; in this case the iso-workload reported would be data at 130 W, from each SL, ALT1 and ALT16. TTSCE was performed at rest and during the last min of each 3 min workload with subjects positioned on the cycle ergometer in the forward leaning aerobar position to facilitate obtaining a clear apical, four-chamber view.

Pulmonary artery systolic pressure and cardiac output

Following each saline contrast injection, pulmonary artery systolic pressure (PASP) was assessed from the peak velocity of the tricuspid regurgitation using Doppler ultrasound (Sonosite Micromaxx, Bothell, WA, USA) and applied to the modified Bernoulli equation ($4v^2 + P_{RA}$), where v is velocity of the tricuspid regurgitation velocity envelope and P_{RA} is right atrial pressure, according to the guidelines of the American Society for Echocardiography (29, 52, 70). A small volume (<0.5 ml) of air agitated with 3 ml of sterile saline was injected and used to help delineate the tricuspid regurgitation velocity envelope. Cardiac output (Q_T) was calculated as before (58) with heart rate obtained from the ECG and stroke volume estimates derived from intra-arterial blood pressure tracings obtained via a saline filled

transducer (Utah Medical, Salt Lake City, UT, USA) positioned at heart level and attached to the radial artery catheter (6, 59).

Arterial blood gases, body temperature and blood lactate

At rest and at the end of each 3 min submaximal workload, a 3 ml radial artery blood sample was drawn anaerobically over 10-15 sec into a heparinized syringe and rapidly analyzed in duplicate (or triplicate if time permitted) for arterial PO_2 (PaO_2), arterial PCO_2 ($PaCO_2$), and pH with a blood-gas analyzer calibrated daily with tonometered whole blood (Siemens RAPIDLab 248, Erlangen, Germany). Arterial blood gases were corrected for body temperature (10, 27, 56) based on readings from the ingested core temperature pill. Arterial O_2 saturation (SaO_2) and hemoglobin (Hb) were measured with CO-oximetry (Radiometer OSM3, Copenhagen, Denmark). Hematocrit was analyzed in triplicate at rest and in single measurements for each workload using the microcapillary tube centrifugation method (M24 Centrifuge, LW Scientific, Lawrenceville, GA, USA). Blood lactate was analyzed in duplicate using the Lactate Plus hand held meter and lactate test strips (Nova Biomedical, Waltham, MA, USA).

Calculations

Alveolar PO_2 (PAO_2) was calculated using the ideal gas equation, as before (11, 13, 36), using temperature corrected $PaCO_2$, and a respiratory quotient (RER) from a 15 sec average of metabolic data corresponding to the time and duration of the arterial blood draw:

$$P_AO_2 = [(P_B - e^{0.05894809 \times T_B + 1.689589}) \times FIO_2] - PaCO_2 \times \left[FIO_2 + \frac{(1 - FIO_2)}{RER}\right]$$

where T_B is core body temperature for temperature correction of water vapor pressure, RER is the respiratory exchange ratio (VCO_2/VO_2), and P_B is barometric pressure that was measured daily (Greisinger electronic, GPB 3300). Pulmonary gas exchange efficiency (A-aDO_2) was determined at rest and during exercise as the difference between the temperature corrected PaO_2 and corresponding PAO_2.

Measures of O_2 content were calculated from the standard content equation:

$$O_2\ \text{Content} = \left[1.39 \times \text{Hb} \times \left(\frac{SO_2}{100}\right)\right] + (0.003 \times PO_2)$$

using an O_2 carrying capacity of 1.39 ml O_2/g Hb and directly measured Hb (g Hb/dL). For arterial O_2 content (CaO_2) SO_2 represents CO-oximetry measured arterial O_2 saturation (SaO_2) and temperature corrected PaO_2. For end-capillary O_2 content ($Cc'O_2$) SO_2 represents the end-capillary O_2 saturation ($Sc'O_2$) calculated from the Kelman equation (28) assuming complete alveolar-capillary O_2 equilibration such that end-capillary PO_2 ($Pc'O_2$) was equal to PAO_2. Mixed venous O_2 content (CvO_2) was calculated using the Fick principle of mass balance ($VO_2 = Q_T \times (CaO_2 - CvO_2)$) using measured CaO_2, VO_2, and an estimate of total cardiac output (Q_T) as described above.

The fraction of venous admixture (Q_{VA}/Q_T) accounting for the entirety of the A-aDO_2 was calculated from the shunt equation (5) using the previously calculated O_2 content values:

$$\frac{Q_{VA}}{Q_T} = \frac{Cc'O_2 - CaO_2}{Cc'O_2 - C\bar{v}O_2}$$

Alveolar ventilation (V_A) was calculated using the measured VCO_2 and temperature corrected $PaCO_2$:

$$V_A = \frac{(VCO_2 \times 863)}{PaCO_2}$$

Statistical Analyses

All statistical calculations were made using GraphPad Prism statistical software (v. 5.0d) and significance was set to $p < 0.05$. In both the PFO− and PFO+ groups, measured and calculated physiologic variables were compared across time points (e.g., SL, ALT1 and ALT16) using a one-way ANOVA. A Newman-Keuls multiple comparison post-hoc test was used to determine specific pairwise differences between groups and time points. Comparisons were determined *a priori* and performed two times (at rest and during exercise) in the PFO− and PFO+ groups. Differences in measured and calculated variables, between the PFO− and PFO+ groups at rest and during exercise were assessed using an unpaired t-test with Welch’s correction.

RESULTS

Overview

Baseline values for anthropometric, exercise, hematologic and pulmonary function data at SL for the PFO− (*n* = 10) and PFO+ (*n* = 11) groups are presented in **Table 1**. Cardiopulmonary data at rest and during exercise for SL, ALT1 and ALT16 for the PFO− and PFO+ groups are presented in **Table 2** and **Table 3**. The mean iso-workload in the PFO− and PFO+ groups was 150 ± 24 W and 136 ± 28 W, respectively, which was not different between groups. The presentation of results will sequentially describe data collected at rest and during exercise at SL, ALT1 and ALT16 in the PFO− and PFO+ groups.

Anthropometric, exercise, hematologic, and pulmonary function data

No differences were observed between PFO− and PFO+ groups in baseline anthropometric, exercise, or hematologic variables at SL **(Table 1)**. The greater number of females in the PFO+ group (*n* = 7) explains the observed differences in absolute pulmonary function values and results from the known differences in absolute lung volumes between males and females (21). However, when the pulmonary function data were expressed as percent predicted, there were no differences. Of note, preliminary analyses between male and female subjects revealed no differences other than a larger CaO_2 in males as a result of increased Hb across SL, ALT1 and ALT16 (57).

Sea Level (SL)

Pulmonary gas exchange efficiency. Pulmonary gas exchange efficiency (A-aDO_2) in the PFO− and PFO+ groups at rest **(Figure 1A)** and during exercise **(Figure 1B)** was similar.

Acute ascent to 5260 m (ALT1)

Upon arrival to 5260 m the cardiopulmonary and respiratory responses between the PFO− and PFO+ groups at rest and during exercise were similar. The A-aDO_2 increased ~2-fold compared to SL in both

the PFO− and PFO+ groups at rest **(Figure 1A)** and during exercise **(Figure 1B)**. Similarly, PAO_2, PaO_2, $PaCO_2$, and SaO_2 between the PFO− and PFO+ groups at rest **(Figure 1C, E, G, I)** and during exercise **(Figure 1D, F, H, J)** were not different. V_E and V_A were both increased compared to SL in the PFO− and PFO+ groups, and there was no difference in V_E or V_A between groups **(Table 2 and 3)**. Additionally, the change in resting V_E from SL to ALT1 relative to the change in SaO_2 **(Figure 2)** was not different between the PFO− and PFO+ groups.

Acclimatization to 5260 m (ALT16)

Pulmonary gas exchange efficiency. Unlike the PFO− group, the A-aDO_2 at rest **(Figure 1A)** and during exercise **(Figure 1B)** in the PFO+ group did not improve (i.e., decrease) compared to ALT1. Furthermore, although there was only a trend for the A-aDO_2 to be greater in the PFO+ group compared to the PFO− group (p = 0.063) at rest **(Figure 1A)**, the A-aDO_2 was significantly greater in the PFO+ group compared to the PFO− group during exercise **(Figure 1B)**. This difference in pulmonary gas exchange efficiency between the PFO− and PFO+ groups at ALT16 can also be illustrated by calculating the difference in the total Q_{VA}/Q_T required to account for the entire A-aDO_2 for each group between ALT1 and ALT16 **(Figure 3)**. From ALT1 to ALT16 at rest, the PFO− group showed a ~21% reduction in calculated Q_{VA}/Q_T, which was greater than the ~13% reduction in the PFO+ group **(Figure 3)**. Similarly, from ALT1 to ALT16 during exercise, calculated Q_{VA}/Q_T in the PFO− group decreased by ~18%, which was also greater than the ~11% decrease in the PFO+ group **(Figure 3)**.

Ventilatory acclimatization and arterial blood gases. Importantly, both the PFO− and PFO+ groups demonstrated a reduction in $PaCO_2$ and an increase in PaO_2 compared to ALT1, consistent with acclimatization to high-altitude **(Figure 1E and G)**. That said, the PFO+ group had no improvement in A-aDO_2 from ALT1 to ALT16, and they demonstrated a less pronounced degree of ventilatory acclimatization compared to the PFO− group. Only the PFO− group increased V_A at rest at ALT16 compared to ALT1, and V_A was less in the PFO+ group compared to the PFO− group at rest at ALT16

(Table 2). Consequently, the PFO+ group had reduced resting PAO_2, PaO_2, SaO_2 and increased $PaCO_2$ compared to the PFO− group **(Figure 1C, E, G, I)** at rest at ALT16, and both PAO_2 and SaO_2 had not improved after acclimatization compared to ALT1 **(Figure 1C and I)**. This reduced degree of ventilatory acclimatization is also illustrated by the change in resting V_E from SL to ALT16 relative to the change in SaO_2 not increasing at ALT16 compared to ALT1 in the PFO+ group **(Figure 2)**. This contrasts with the PFO− group who showed an increase in the change in resting V_E from SL to ALT16 relative to the change in SaO_2 at ALT16 compared to ALT1 **(Figure 2)**.

The difference in V_A between the PFO− and PFO+ groups at ALT16 was present only at rest and not during exercise at ALT16. Nevertheless, only the PFO− group increased PAO_2 **(Figure 1D)** and decreased $PaCO_2$ **(Figure 1H)** during exercise at ALT16 compared to ALT1. Both the PFO− and PFO+ groups increased PaO_2 and SaO_2 during exercise at ALT16 compared to exercise at ATL1; however, during exercise at ALT16, PaO_2 and SaO_2 in the PFO+ group were lower compared to the PFO− group **(Figure 1F and J)**.

Hemoglobin and hematocrit. Hb and Hct were less in the PFO+ group compared to the PFO− group and only the PFO− group increased Hb and Hct compared to ALT1. However, Hb mass increased compared to SL in 19/21 subjects and the 2 subjects who lacked an increase in Hb mass were PFO+ females (53). Statistical analysis of the PFO+ group without these two subjects shows no differences between the PFO− and PFO+ groups in Hb ($p = 0.20$) or Hct ($p = 0.21$) at ALT16; therefore, in both the PFO− and PFO+ groups Hb and Hct increased compared to ALT1.

DISCUSSION

The primary purpose of this study was to investigate the effect of a PFO on pulmonary gas exchange efficiency at rest and during exercise at sea level (SL), after rapid transport to 5260 m (ALT1) and after living at 5260 m for 16 days (ALT16) in healthy, PFO− (n = 10) and PFO+ (n = 11) sea level natives. The novel findings in this study are: 1) pulmonary gas exchange efficiency did not improve at rest or during exercise after acclimatization to 5260 m in the PFO+ group; and 2) there was a reduced degree of ventilatory acclimatization to 5260 m in the PFO+ group.

Sea Level (SL)

Three distinct factors can impair pulmonary gas exchange efficiency: 1) alveolar ventilation-to-perfusion (V_A/Q) inequality; 2) incomplete end-capillary O_2 diffusion equilibration; and 3) any source of right-to-left shunt [extrapulmonary shunt (e.g. venous blood from the bronchial and Thebesian circulations), intracardiac shunt (e.g. blood flow through a PFO), and intrapulmonary shunt]. Although this study did not directly assess contributions from V_A/Q inequality or diffusion limitation, previous work suggests that diffusion limitation represents a minimal contribution to the A-aDO_2 during submaximal exercise (VO_2 <2.0 L/min) in healthy humans at SL (20, 25, 49, 60, 62) such that the majority of the A-aDO_2 is explained by V_A/Q inequality and right-to-left shunt. Accordingly, during iso-workload exercise (VO_2 ~2.0 L/min) in our healthy subject population, at SL V_A/Q inequality and right-to-left shunt were likely the predominant contributors to the measured A-aDO_2. In both PFO− and PFO+ subjects this would include extrapulmonary shunt, and intrapulmonary shunt, if any. However, subjects in the PFO+ group also have an additional source of shunt, which is intracardiac shunt via the PFO.

Right-to-left blood flow through the PFO is dependent upon right atrial pressure exceeding left atrial pressure, which can occur transiently during the normal respiratory cycle, likely at end inspiration when systemic venous return is augmented by reduced intrathoracic pressure (16, 69). Therefore, during normal respiration, right-to-left blood flow through the PFO would be expected to be intermittent and

variable in volume. In the current study the A-aDO_2 was not different between the PFO− and PFO+ groups at rest or during exercise at SL, suggesting that the degree of blood flow through the PFO was not great enough to impact the A-aDO_2 at SL.

Acute Ascent to 5260 m (ALT1)

At 5260 m (P_B ~406 mm Hg) inspired PO_2 is lowered to ~75 mm Hg, significantly reducing PAO_2, and reducing the contribution from V_A/Q inequality on the A-aDO_2 while the contribution of diffusion limitation on the A-aDO_2 increases (46, 47, 66). The effect that a given volume of right-to-left shunt has on the A-aDO_2 is also lessened in hypoxia due to the difference between mixed venous PO_2 (PvO_2) and PaO_2 becoming less. For this reason, if the shunt fraction via the PFO was constant from SL to ALT1, this additional 0.5-2.0% shunt (as it was calculated to be at SL) would account for between 1-3 mm Hg of the measured A-aDO_2 at ALT1. Therefore, it should not be surprising that at ALT1 the PFO− and PFO+ groups had similar degrees of pulmonary gas exchange impairment both at rest and during exercise **(Figure 1B)**.

Previous work has suggested the presence of PFO may facilitate an exaggerated pulmonary hypertensive response to high-altitude, thereby predisposing these subjects to the development of HAPE (6). In that work, of the 16 HAPE susceptible subjects studied, 11 were PFO+, and PASP was found to be 57 ± 12 mm Hg at 4550 m. In the current work, at a similar altitude, PASP in the PFO+ group was ~32 ± 6 mm Hg. Although not conclusive, our data suggest that HAPE susceptibility may depend more on an exaggerated pulmonary vascular response to hypoxia rather than on the presence of PFO.

Acclimatization to 5260 m in PFO− and PFO+ subjects at (ALT16)

Acclimatization to hypobaric hypoxia is characterized by a multitude of physiologic adaptations, notably a time-dependent increase in ventilation (65). Compared to acute hypoxia, ventilatory acclimatization helps to increase CaO_2 by increasing PAO_2 and the driving gradient for O_2 diffusion at the alveolar-capillary interface, increasing PaO_2 and therefore, SaO_2. Consequently, compared to acute hypoxia the

further increase in V_A, and therefore PAO_2 with acclimatization would theoretically reduce the relative contributions from V_A/Q inequality and diffusion limitation while increasing the relative contribution of right-to-left shunt on the A-aDO_2. Indeed, an increase in V_A should equate to improved V_A/Q matching by way of lessening potential disparities in the PO_2 between alveoli (15, 48, 55). Diffusion limitation would theoretically also be reduced compared to ALT1 by way of an increased driving gradient for O_2 diffusion (63), increased PvO_2, and a potential improvement in diffusing capacity for O_2 (1). Lastly, a given volume of right-to-left shunt would have a greater effect on the A-aDO_2 due to the difference between PvO_2 and PaO_2 increasing at ALT16 compared to ALT1.

Rest. Accordingly, absence of an increased V_A at rest at ALT16 in the PFO+ group may partially explain the absence of a reduction in A-aDO_2 compared to ALT1. The less pronounced degree of ventilatory acclimatization in the PFO+ group corresponded to a lower PAO_2, which, compared to the PFO− group, may increase the relative contribution and potential for V_A/Q inequality and, particularly diffusion limitation to contribute to the A-aDO_2. Additionally, continued right-to-left shunt via the PFO could have also contributed to the lack of improvement in A-aDO_2 at rest at ALT16. However, the effect of this right-to-left shunt via the PFO, although increased compared to ALT1, would still likely be minimal considering the magnitude of the increase in PaO_2 from ALT1 to ALT16 (40 ± 5 mm Hg at ALT1 vs. 46 ± 3 mm Hg at ALT16). Nevertheless, bubble scores were increased in the PFO+ group at rest at ALT16 and although this neither quantifies blood flow nor strictly reflects blood flow through the PFO, it supports the occurrence of right-to-left shunt via the PFO in the PFO+ group. Conversely, in the PFO− group PaO_2 increased from 40 ± 4 mm Hg at ALT1 to 53 ± 4 mm Hg at ALT16, approximately twice as much as of the PFO+ group (p = 0.0003). Importantly, small changes in PaO_2 in this range on the oxygen-hemoglobin dissociation curve correspond to large changes in SaO_2, and thus CaO_2. Indeed, had PaO_2 increased in the PFO+ group to the same degree as it did in the PFO− group, SaO_2 in the PFO+ group would have increased from ~83% to ~88%, corresponding to CaO_2 increasing from ~18 mL O_2/dL blood to ~19 mL O_2/dL blood.

Why the PFO+ group had a lesser degree of ventilatory acclimatization compared to the PFO− group remains unknown, although we speculate that this may actually represent a beneficial response to hypoxia in subjects with an intracardiac right-to-left shunt (i.e., the PFO+ group). Increasing ventilation and therefore raising PAO_2 would increase PaO_2 in PFO− subjects to a greater extent than PFO+ subjects, due to the continued presence of right-to-left shunt via the PFO. Indeed, perfusion without ventilation defines a right-to-left shunt. Therefore, the metabolic demand associated with increasing ventilation would potentially benefit PFO+ subjects to a lesser degree in terms of raising PaO_2, compared to PFO− subjects. Furthermore, considering pH and temperature were not different between the PFO− and PFO+ groups, the lower V_A in the PFO+ group resulted in a higher $PaCO_2$ and thus, a right-shifted oxygen-hemoglobin dissociation curve. Estimating this shift based off of the calculated p50 values using the Hill equation (23) (Hill coefficient = 2.7), the PFO+ group had a higher p50 (29.1 ± 0.7 mmHg) compared to the PFO− group (28 ± 1 mmHg), $p = 0.036$. This would facilitate the unloading of O_2 at the tissue, which would be beneficial for PFO+ subjects considering their impaired ability to raise PaO_2 and thus SaO_2, due to right-to-left shunt via the PFO. Interestingly, in animals with an intracardiac right-to-left shunt and in children with cyanotic congenital heart disease, the presence of a right-shifted oxygen-hemoglobin dissociation curve has also been hypothesized to be a possible compensatory mechanism for facilitating O_2 unloading and therefore reducing tissue hypoxia in conditions when increasing ventilation would be ineffective in increasing the PaO_2 of the shunted blood (44, 67).

Exercise. During exercise the A-aDO_2 is greater in acute hypoxia compared to SL and decreases following acclimatization to high-altitude compared to acute hypoxia (4, 8, 9, 38), yet the cause of this subsequent improvement in A-aDO_2 remains speculative. Considering the sample sizes of these prior studies (n = 6-10), statistically we would expect each study to have 2-4 PFO+ subjects, and it is unknown to what extent such subjects could potentially have influenced the findings in these previous studies. In the current study when the A-aDO_2 data from the PFO− and PFO+ groups is pooled, as previously shown, pulmonary gas exchange efficiency improves following acclimatization to hypobaric

hypoxia **(Figure 4)**. However, by prospectively identifying PFO− and PFO+ subjects, the current work suggests that this reduction in the A-aDO_2 after acclimatization was not present in the PFO+ subjects in our study. As previously discussed, right-to-left blood flow through the PFO would be expected to intermittent and variable in volume and dependent on a sufficient pressure gradient between the right and left atria. Using pulmonary artery systolic pressure (PASP) as an estimate for this potential pressure gradient, PASP was higher at rest and during exercise at ALT16 in PFO+ and PFO- subjects. Thus, there was a potential for greater blood flow across a PFO at ALT 16.

In contrast to rest, V_A and PAO_2 were not different between the PFO− and PFO+ groups during exercise at ALT16. This suggests that contributions from V_A/Q inequality and diffusion limitation to the A-aDO_2 during exercise may be relatively equal between the PFO− and PFO+ groups. However, while not directly measured, we cannot rule out the possibility that differences between the PFO− and PFO+ groups in terms of V_A/Q inequality and diffusion limitation existed. Nevertheless, the intracardiac right-to-left shunt via the PFO in the PFO+ group could have also contributed to the lower PaO_2 in the PFO+ group **(Figure 1E)**, and therefore, contributed to the lack of improvement in A-aDO_2 compared to ALT1 and significantly greater A-aDO_2 compared to the PFO− group at ALT16 **(Figure 1B)**. The calculated volume of venous admixture required to account for the difference in A-aDO_2 during exercise at ALT16 between the PFO− and PFO+ groups was ~7%. This ~7% difference between the PFO− and PFO+ groups includes all sources of venous admixture, yet this doesn't preclude the possibility that the intracardiac right-to-left shunt in the PFO+ group was contributing to the lack of improvement in pulmonary gas exchange efficiency expected to occur with acclimatization to high-altitude.

Although our exercise data at ALT16 indicate worse pulmonary gas exchange efficiency in the PFO+ group, this did not translate into differences in functional exercise capacity, that have been described previously (57). However, neither group had an A-aDO_2 >25 mm Hg at this submaximal workload, and therefore it is unlikely that exercise capacity would be limited due to pulmonary gas exchange inefficiency. Alternatively, the lack of functional difference between groups may also be the

result of the right-shifted oxygen-hemoglobin dissociation curve that facilitated the unloading of oxygen despite the fact that pulmonary gas exchange efficiency did not improve with acclimatization and ventilatory acclimatization was less than PFO− subjects.

Limitations

Summary

The current study aimed to assess the impact of a PFO on pulmonary gas exchange efficiency at rest and during exercise at SL, with acute transport to 5260 m, and after living at 5260 m for 16 days. We identified an improvement in pulmonary gas exchange efficiency with acclimatization to high-altitude similar to previous investigations; however, this finding was not present in PFO+ subjects. The additional source of intracardiac right-to-left shunt in PFO+ subjects may be sufficient to explain their impaired pulmonary gas exchange efficiency at rest and during submaximal exercise compared to PFO− subjects at SL. However, the contribution of this right-to-left shunt to pulmonary gas exchange efficiency is reduced at altitude and may only partially explain the lack of improvement in pulmonary gas exchange efficiency at ALT16. PFO+ subjects demonstrated a less pronounced degree of ventilatory acclimatization to 5260 m, concomitant with a greater A-aDO_2 and lower PaO_2 and SaO_2. Although future work is needed to corroborate these findings, we speculate that this reduction in ventilatory acclimatization may be beneficial in PFO+ subjects by limiting the metabolic cost of hyperventilation, which would not effectively increase PaO_2 in the presence of a right-to-left shunt. Ultimately, a more effective strategy may be to ventilate less, resulting in a right-shifted oxygen-hemoglobin dissociation curve that facilitates O_2 unloading at the tissue when O_2 loading is hindered by the presence of an intracardiac right-to-left shunt.

Acknowledgements: This paper is part of a series titled “AltitudeOmics” that together represent a group of studies that explored the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations invested enormous time and resources to make this project a success. Foremost, the study was made possible by the tireless support, generosity and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, Andrew T. Lovering, Andrew W. Subudhi and Robert C. Roach. A complete list of other investigators on this multinational, collaborative effort involved in development, subject management and data collection, supporting industry partners, and people and organizations in Bolivia that made AltitudeOmics possible is available in the project overview paper in this series (57). The authors are extremely grateful to Jui-Lin “Mickey” Fan and Nicolas Bourdillon for their invaluable assistance with temperature recording and acquisition of metabolic data for this study. The authors would also like to extend our gratitude to Benjamin Ryan, Nadine Wachsmuth, Jenna Bucher, and See Eun Kim for technical assistance with data collection. We also wish to thank Joseph Duke for assistance with statistical analyses.

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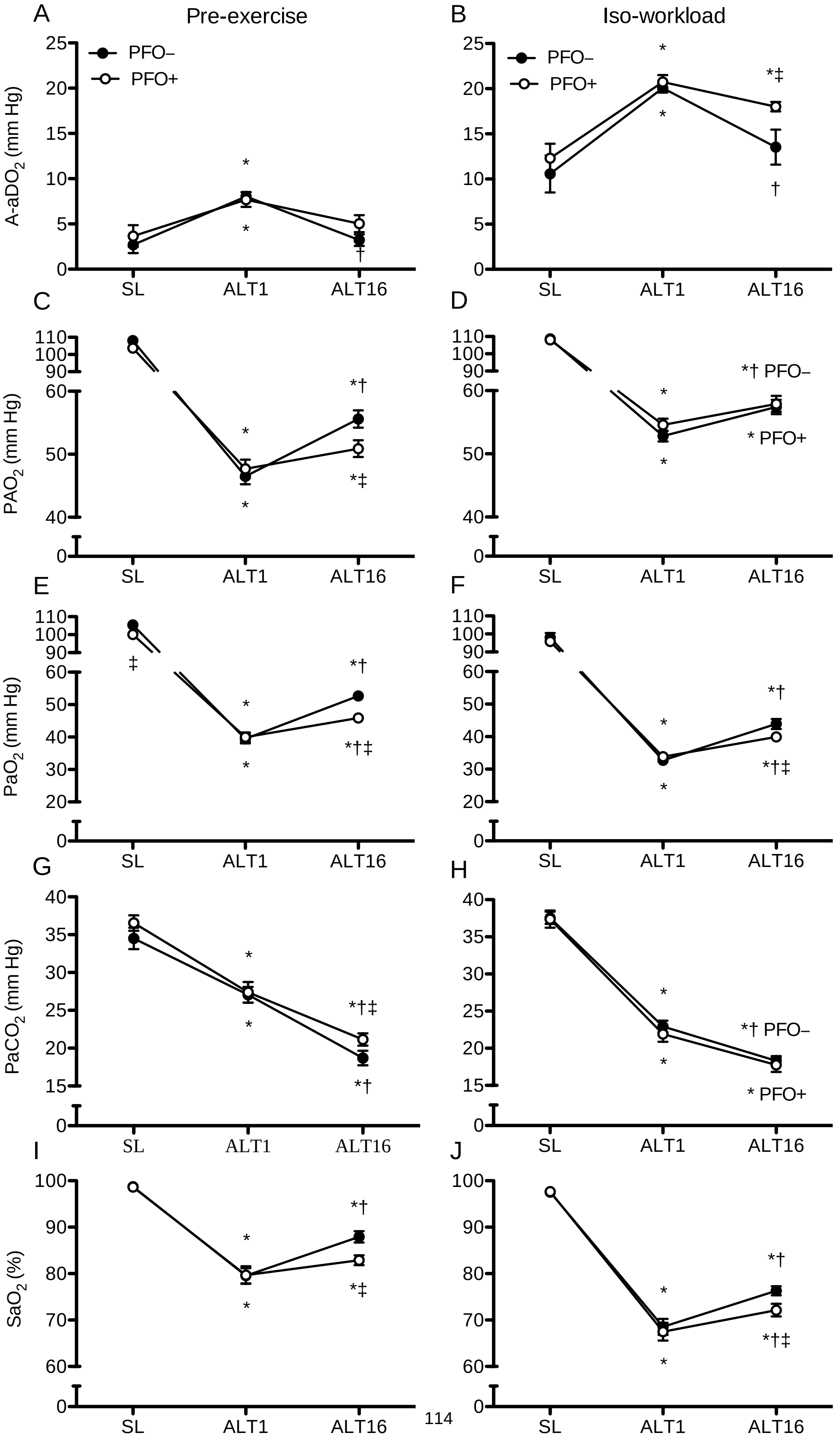
Tables

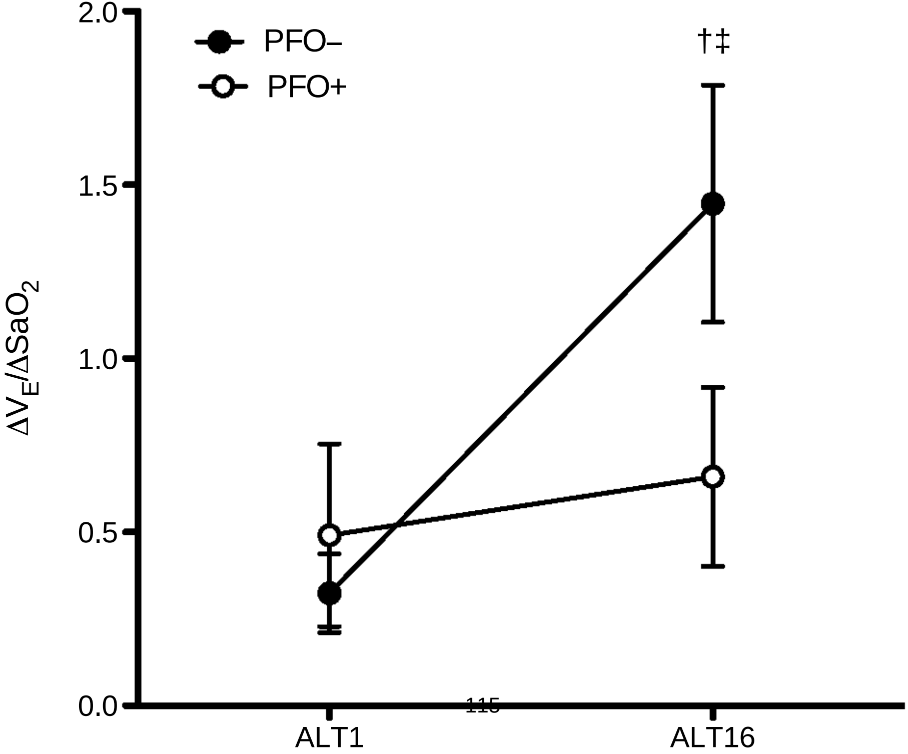
Table 1. *Anthropometric, exercise, hematologic and pulmonary function data.*

	PFO−, n=10	PFO+, n=11
Age, yr	21.1 ± 1.4	20.6 ± 1.6
Sex, (female)	2	7
Height, cm	178.4 ± 6.1	173.4 ± 8.8
Weight, kg	71.5 ± 8.4	68.1 ± 9.7
VO_{2peak}, $mL \cdot kg^{-1} \cdot min^{-1}$	46.8 ± 7.1	44.0 ± 6.2
Peak power output, W	311 ± 67	268 ± 64
Ferritin, $ng \cdot ml^{-1}$	61.3 ± 30.6	44.0 ± 32.7
Iron, $\mu g \cdot dL^{-1}$	129.5 ± 47.9	134.1 ± 64.1
FVC, L	5.48 ± 0.92	4.73 ± 1.04‡
FVC, %p	100.0 ± 11.0	100.4 ± 11.3
SVC, L	5.61 ± 1.08	5.01 ± 1.18
SVC, %p	102.4 ± 13.5	105.6 ± 11.7
FEV_1, L	4.62 ± 0.60	4.07 ± 0.77‡
FEV_1, %p	101.3 ± 9.0	101.9 ± 7.9
FEV_1/FVC	85.4 ± 9.8	86.4 ± 5.3
FEV_1/FVC, %p	100.9 ± 11.6	100.8 ± 6.1
$FEF_{25\text{-}75}$, $L \cdot sec^{-1}$	4.79 ± 1.37	4.51 ± 0.84
$FEF_{25\text{-}75}$, %p	100.1 ± 28.5	105.1 ± 15.3
TLC, L_{pleth}	7.16 ± 1.07	6.28 ± 1.33
TLC, %p	104.9 ± 8.2	103.3 ± 11.4
DL_{CO}, $mL \cdot min^{-1} \cdot mm\ Hg^{-1}$	43.06 ± 8.74	33.25 ± 10.33‡
DL_{CO}, %p	130.6 ± 21.1	110.1 ± 19.4‡
DL_{CO}/V_A	6.00 ± 0.71	5.61 ± 1.03
DL_{CO}/V_A, %p	124.1 ± 17.5	113.2 ± 21.4

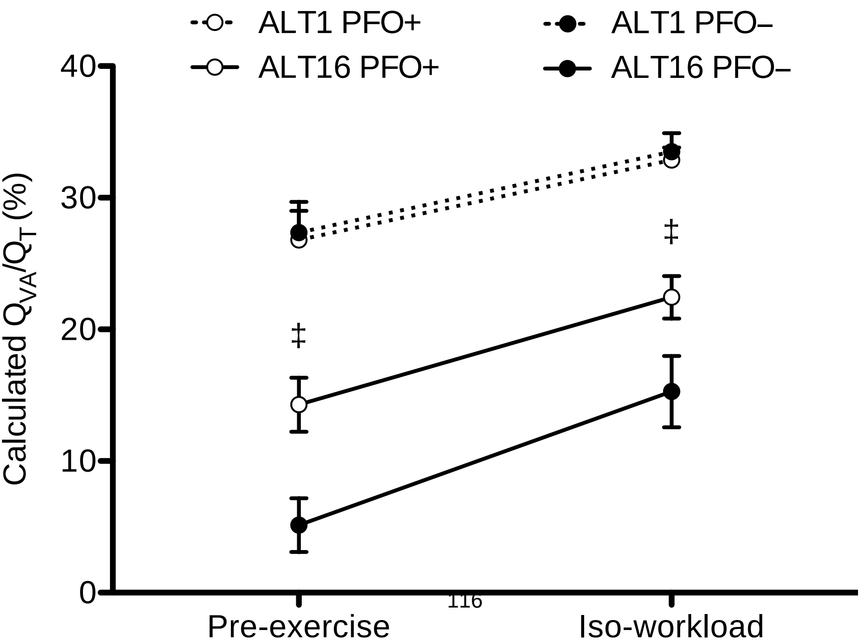
Values are mean ± standard deviation. FVC, forced vital capacity; SVC, slow vital capacity; FEV_1, forced expiratory volume in 1 s; $FEF_{25\text{-}75}$, forced expiratory flow from 25 to 75% of FVC; TLC, total lung capacity; DL_{CO}, diffusion capacity of carbon monoxide; DL_{CO}/V_A, DL_{CO}/alveolar volume; %p, % predicted; ‡ $p < 0.05$ compared to PFO−.

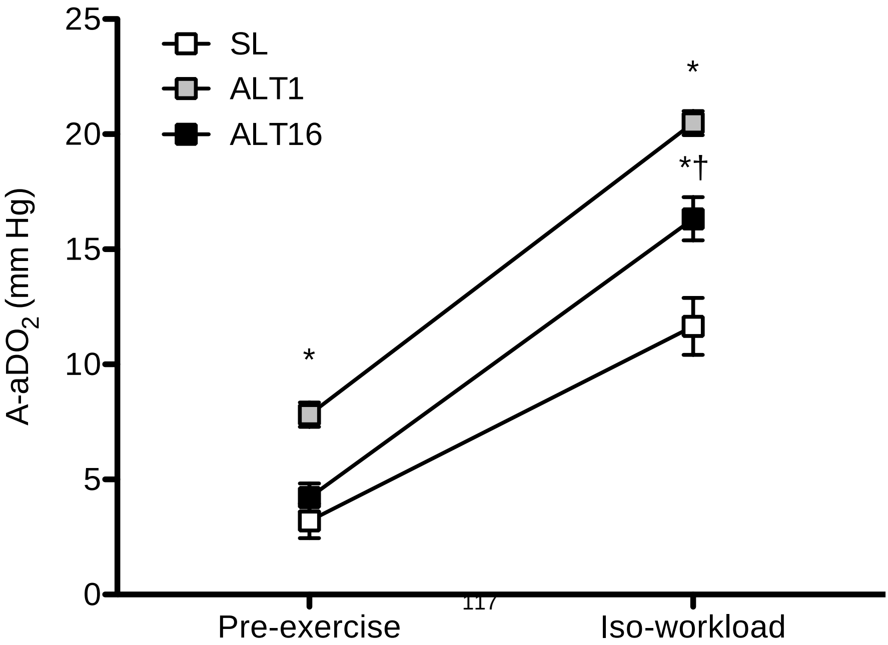


A
Pre-exercise
B
Iso-workload
PFO–
PFO+
A-aDO2 (mm Hg)
PAO2 (mm Hg)
PaO2 (mm Hg)
PaCO2 (mm Hg)
SaO2 (%)
SL
ALT1
ALT16
C
D
E
F
G
H
I
J
*† PFO–
* PFO+



PFO–
PFO+
†‡
2.0
1.5
1.0
0.5
0.0
$\Delta V_E/\Delta SaO_2$
ALT1
ALT16


ALT1 PFO+
ALT1 PFO–
ALT16 PFO+
ALT16 PFO–
Calculated Q_{VA}/Q_T (%)
40
30
20
10
0
‡
‡
Pre-exercise
Iso-workload


25
20
15
10
5
0
A-aDO2 (mm Hg)
SL
ALT1
ALT16
*
*
*†
Pre-exercise
Iso-workload

Adenosine-induced erythrocyte oxygen release: a key mechanism for human hypoxia adaptation

By

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Running Title: Adenosine-regulated erythrocyte function in hypoxia

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ABSTRACT

High altitude sickness (HAS) is a dangerous and urgent condition given its association with hypoxia-induced multiple tissue damage and even sudden death. However, specific means to prevent or treat HAS are limited due to lack of understanding of the molecular basis underlying adaptation to high altitude. Here we report that in 19 young healthy human volunteers, the level of circulating adenosine and the activity of soluble CD73 (an ectonucleotidase that generates extracellular adenosine) increased within two hours of arrival at 5260 m, and further increased following 16 days at this altitude. Mouse genetic studies demonstrated that elevated CD73 contributes to increased circulating adenosine and that CD73-mediated induction of adenosine protects hypoxia-induced tissue damage. Mechanistically, we revealed that elevated circulating adenosine signaling through the erythrocyte A2B adenosine receptor (ADORA2B) resulted in the phosphorylation and activation of AMP-activated protein kinase (AMPK), a well-known cellular energy sensor. Activated AMPK phosphorylated 2,3-bisphosphoglycerate (2,3-BPG) mutase, leading to increased production of 2,3-BPG, a negative allosteric regulator of hemoglobin-O_2 binding affinity, thereby triggering O_2 release. Preclinical studies showed that the pharmacologic activation of AMPK by metformin, a FDA approved drug, significantly reduced hypoxia-induced tissue damage by inducing 2,3-BPG

production and increased O_2 delivery to local tissues. Human translational studies validated the mouse findings by revealing that high altitude significantly increased phosphorylation of AMPK and 2,3-BPG mutase in the human volunteers. Overall, our studies have revealed that erythrocyte adenosine signaling coupled with activation of AMPK is a novel mechanism underlying human adjustment to hypoxia, suggesting new and innovative anti-hypoxia therapeutic targets.

INTRODUCTION

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Altitude hypoxia is a challenging condition due to limited oxygen (O_2) availability. The ability to adjust to altitude hypoxia (around 5000 m where ambient O_2 pressure is half that of sea level) varies considerably among individuals. Failure to adapt to altitude hypoxia results in high altitude sickness (HAS). HAS is frequently associated with poor exercise performance, severe headache, dizziness and vomiting. Without intervention, it can progress to pulmonary edema, stroke and death[1, 2]. Current strategies to manage HAS are poor due to lack of fundamental understanding of mechanisms for human acclimatization to high altitude hypoxia. Here, we sought to determine the molecules and signaling pathways responsible for high altitude hypoxia adaptation. Such findings are likely to provide us a better understanding of the molecular basis underlying acute altitude hypoxia adaptation and thereby addresses the challenge of identifying novel approaches for the safe and effective

prevention and treatment of HAS.

The erythrocyte is the most abundant circulating cell type and has the responsibility to deliver O_2 to peripheral tissues. One of best studied molecules regulating O_2 release from erythrocytes is 2,3-biphosphoglycerate (2,3-BPG), an erythrocyte-specific allosteric modulator that binds to the central water cavity of the hemoglobin tetramer, resulting in decreased Hb-O_2 binding affinity[3-5]. Intriguingly, several early human studies showed that in responses to high altitude, normal individuals adaptively increase the level of erythrocyte 2,3-BPG, suggesting that elevated erythrocyte 2,3-BPG levels is likely an important adaptive response to prevent hypoxia-induced HAS[6]. However, the specific molecules and signaling pathways responsible for increased erythrocyte 2,3-BPG at high altitude have not been identified. Therefore, the identification of such pathways could play an important role in identifying therapeutic approaches to high altitude adaption.

Adenosine is well-known to orchestrate a physiological response to tissue injury and hypoxia. However, a role for adenosine in the physiological response to high altitude hypoxia has not been investigated. Extracellular adenosine is induced under hypoxia conditions by two ecto-nucleotidases, CD39 and CD73 that sequentially convert extracellular ATP to AMP and AMP to adenosine. Circulating adenosine exerts its function by the activation of four G-protein coupled receptors on multiple cell types[7, 8]. Intriguingly, recent studies demonstrated that high circulating adenosine is

detrimental to individuals with sickle cell disease (SCD) by activating the erythrocyte A_{2B} adenosine receptor (ADORA2B) resulting in the induction of 2,3-BPG. The elevated 2,3-BPG promotes O_2 release, increases deoxyHbS polymerization, thereby triggering erythrocyte sickling, the central pathogenic process of the disease[9]. Thus, although adenosine induced accumulation of 2,3-BPG is detrimental for individuals with SCD, it may be beneficial to normal individuals facing hypoxic conditions. Here we conducted human high altitude studies along with genetic and pharmacological studies in mice to investigate the functional role of adenosine signaling in erythrocytes as a physiological mechanism underlying adaption to high altitude.

In this study, we report for the first time that plasma adenosine levels and soluble CD73 activity were significantly elevated in 19 lowland volunteers when they were brought to 5260 m. The increase was observed within two hours of arrival at 5260 m and further increased by day 16 at high altitude. Genetic studies with mice demonstrated that CD73 is essential for increased production of circulating adenosine and that enhanced adenosine signaling via ADORA2B on erythrocytes induced 2,3-BPG production, promoting O_2 release and thereby protected against hypoxia-induced tissue damage in mice. Mechanistically, we determined that AMPK, an energy sensor, is a key enzyme underlying ADORA2B-induced erythrocyte 2,3-BPG production and O_2 release. Preclinical studies showed that pharmacologic enhancement of AMPK activity promoted 2,3-BPG induction, triggered O_2 release and thereby prevented hypoxia-induced tissue injury in mice. Human translational studies confirmed mouse studies showing that activation of AMPK was associated with high altitude

adaption in the human volunteers. Overall, both human and mouse studies add a significant new chapter to erythrocyte physiology by revealing altitude dependent adenosine production resulting in the activation of erythrocyte AMPK, subsequently increased erythrocyte 2,3-BPG levels causing elevated O_2 release from Hb. Our results highlight innovative therapeutic opportunities for HAS and hypoxia-induced tissue damage.

RESULTS

Plasma adenosine level and soluble CD73 activity increased proportionately in humans at high altitude

To determine if adenosine is increased in the circulation of humans adjusting to high altitude, we recruited 19 normal lowland volunteers who were examined at sea level and following transport to high altitude. Blood was withdrawn at sea level and within two hours of arrival at an altitude of approximately 5260 meters (ALT1). The results (Fig. 1a) show that the circulating adenosine levels were elevated within two hours of arrival at high altitude compared to sea level and that circulating levels of adenosine were further elevated on ALT16 following an extended stay at 5260 m. Moreover, no significant differences in elevated plasma adenosine levels were observed between males and females (Supplementary Fig.1a-b).

Ecto-5'-nucleotidase (CD73) is an enzyme anchored to the cell surface that plays a key role in the synthesis of extracellular adenosine from AMP. Under certain circumstances the ectoenzyme can be cleaved from the cell surface and exist in the circulation as a soluble nucleotidase. To determine if elevated plasma adenosine is associated with elevated circulating CD73 in high altitude, we measured soluble CD73 activities in normal individuals at sea level and at in response to high altitude. We found that soluble CD73 activity was significantly increased compared to sea level in the human subjects within two hours following arrival at 5260 m (ALT1) and that CD73 levels were further increased following an extended stay at 5260 m (ALT16) (Fig.1b). Furthermore, the increase in plasma adenosine levels was proportional to elevated soluble CD73 activity at ALT16 (Fig.1e). Like adenosine levels, there was no significant difference in elevated CD73 activity between males and females in response to high altitude (Supplementary Fig.1c-d). These results show that plasma adenosine levels increased rapidly in response to high altitude hypoxia and that the increase correlated with that of soluble CD73 activity.

Altitude hypoxia-induced elevated plasma adenosine and soluble CD73 activity correlate to increased erythrocyte 2,3-bisphosphoglycerate (2,3-BPG) levels and O_2 releasing capacity in humans

Next, we validated earlier human studies and found that erythrocyte 2,3-BPG levels and O_2 release capacity (reflected by P50, pressure of O_2 required to achieve 50% Hb-O_2 saturation) were significantly elevated in human subjects within two hours of arrival at 5260 m (ALT1). Additionally,

we found that 2,3-BPG levels and P50 values were increased further after prolonged residence at 5260 m (ALT16) (Fig.1c-d). Additionally, the increase in circulating adenosine levels upon extended stay at 5260 m was matched with a corresponding increase in erythrocyte 2,3-BPG levels and enhanced erythrocyte oxygen release capacity (Fig.1f-g). No differences were noted between male and female subjects (Supplementary Fig. 1e-h). These findings provide evidence that increased plasma adenosine levels are correlated to increased erythrocyte 2,3-BPG levels and O_2 release capacity in humans adapting to high altitude.

Soluble CD73 activity is induced under hypoxia and elevated CD73 is essential for hypoxia-induced production of plasma adenosine and increased erythrocyte 2,3-BPG and O_2 releasing capacity in mice

Our human studies raise the novel and compelling possibilities that 1) elevated soluble CD73 activity is responsible for increased plasma adenosine at high altitude, and 2) increased circulating adenosine plays a beneficial role in the adaptive response to high altitude hypoxia by inducing erythrocyte 2,3-BPG production, resulting in reduced hemoglobin oxygen binding affinity, thereby allowing increased O_2 release to peripheral tissues. Because it is very difficult to test these hypotheses in humans, we used an experimental model in which mice were maintained in an environment of 10% oxygen to mimic the PO_2 conditions at 5260 m. Thus, to determine the role of CD73 in the adaptive response to hypoxia we exposed wild type mice (WT) and CD73-deficient mice (*Cd73*$^{-/-}$) to hypoxia (10% oxygen) for one week. Similar to human high altitude studies, we found that circulating CD73 activity, levels of plasma adenosine, erythrocyte 2,3-BPG and O_2 releasing capacity were

significantly increased in WT mice following 1 week of hypoxia compared to normoxia (Fig. 2a-d). Moreover, the increased plasma adenosine levels correlated well with increased circulating CD73 activity and elevated erythrocyte 2,3-BPG levels (Fig. 2e-f). In contrast, hypoxia-mediated increased levels of plasma adenosine, erythrocyte 2,3-BPG and erythrocyte O_2 releasing capacity were significantly impaired in *Cd73*$^{-/-}$ mice compared to WT mice. These results indicate that circulating CD73 activity is induced in mice by hypoxia as seen in human subjects at high altitude and that CD73 is required for hypoxia-induced plasma adenosine production, elevated erythrocyte 2,3-BPG levels and increased O_2 release capacity from erythrocytes.

CD73-mediated elevated extracellular adenosine protects hypoxia-induced tissue damage in mice

To assess the functional importance of CD73-mediated increased plasma adenosine in hypoxia-induced tissue injury, we measured the tissue hypoxia levels by hypoxia probe in WT mice and C*d73*$^{-/-}$ mice under normoxic conditions and following one week of hypoxia as described above. No hypoxia signals were seen in tissue sections of C*d73*$^{-/-}$ or WT mice under normoxic conditions (Fig. 2g). Histological analysis showed that kidneys, lungs and heart were normal and no obvious damage was observed in C*d73*$^{-/-}$ or WT mice under normoxic conditions (Fig. 2h). However, under hypoxic conditions, immunohistochemical (IHC) analysis with hypoxic probe revealed that multiple organs including kidneys, lungs and hearts displayed mild hypoxic signaling in WT mice (Fig. 2g). In contrast, hypoxia led to severe hypoxia in various organs including kidneys, lungs and hearts of C*d73*$^{-/-}$ mice (Fig. 2g). Image quantification analysis demonstrated that the intensity of hypoxia

probe in the kidneys, lungs and hearts were significantly elevated in C*d73*$^{-/-}$ mice compared to WT mice (Supplementary Fig. 2A-C). Furthermore, histological studies revealed multiple tissue damage including swollen glomeruli, renal tubular edema, pulmonary edema and endovascular injury and increased immune cell infiltration (Fig. 2h). These results show that CD73-mediated elevation of plasma adenosine plays a beneficial role to protect hypoxia-induced multiple tissue damage in mice.

ADORA2B is essential for hypoxia-induced erythrocyte 2,3-BPG and O_2 release capacity to peripheral tissues in mice

A detrimental role for adenosine signaling in erythrocytes of individuals with sickle cell disease was recently reported and shown to result from excessive ADORA2B activation leading to increased 2,3-BPG production, HbS deoxygenation and erythrocyte sickling [9]. However, a beneficial role for adenosine signaling in normal erythrocyte physiology has not been examined. Here we provide evidences from human and mouse studies that; 1) circulating CD73, plasma adenosine, and erythrocyte 2,3-BPG and O_2 releasing capacity were significantly elevated in humans at high altitude and in mice under similar hypoxia (i.e. 10% O_2); 2) CD73 is a key enzyme contributing to hypoxia-induced plasma adenosine levels, erythrocyte 2,3-BPG production and O_2 releasing capacity in mice; and 3) CD73-mediated increased adenosine is beneficial to prevent hypoxia-induced tissue damage in mice. These findings immediately suggest that elevated extracellular adenosine, likely signaling via ADORA2B on erythrocytes, protects hypoxia-induced tissue damage by promoting 2,3-BPG induction and subsequent O_2 release to peripheral tissues. To test this intriguing possibility, we generated mice with erythrocyte specific ablation of A*dora2b* genes (A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$*)* by

mating floxed A*dora2b*$^{f/f}$ mice with the erythropoietin receptor-Cre (E*poR-Cre*$^{+}$) mice containing a transgene expressing Cre recombinase only in the erythroid lineage. First, using western blot analysis, we showed that ADORA2B protein levels of erythrocytes were reduced to levels similar to that of *Adora2b*$^{-/-}$ mice, indicating that we successfully generated mice with erythrocyte specific deletion of ADOAR2B (Supplementary Fig. 3d). Next, to test the functional role of erythrocyte ADORA2B signaling in response to hypoxia, we exposed E*poR-Cre* mice and A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice to normoxia or hypoxia (10% oxygen, similar to that found at 5260 m). The levels of plasma adenosine, erythrocyte 2,3-BPG and P50 were similar in E*poR-Cre*$^{+}$ mice and A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice under normoxia (Fig.3a-c). After 10% O_2 hypoxic treatment for 1 week, plasma adenosine increased to similar levels in EpoR-Cre mice and A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice (Fig.3a). Following one week of hypoxia (10% O_2) the E*poR-Cre*$^{+}$ showed an increase in plasma adenosine levels were associated with increased erythrocyte 2,3-BPG levels and O_2 releasing capacity (Fig. 3a-c). However, hypoxia-induced elevation of erythrocyte 2,3-BPG and P50 were significantly attenuated in A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice compared to Epo-Cre mice (Fig.3a-c), despite the hypoxia-induced elevation in adenosine. Taken together, our studies provide strong genetic evidence that mouse erythrocyte ADORA2B is essential for elevated adenosine-mediated induction of erythrocyte 2,3-BPG levels and O_2 release capacity to peripheral tissues under hypoxic conditions.

Beneficial role of erythrocyte ADORA2B in hypoxia-mediated tissue damage in mice

In order to determine the role of erythrocyte ADORA2B in the tissue protective effect under hypoxia, we assessed the tissue injury level of E*poRCre*$^{+}$ mice and A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice under hypoxia.

We utilized hypoxia probe to assess tissue hypoxia levels in various end organs. Similarly, we found that 1 week 10% O_2 hypoxic treatment led to severe tissue hypoxia in various organs including kidneys, lungs and hearts in A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice, while only a slight hypoxia signal was present in those tissues of E*poR-Cre*$^{+}$ mice (Fig. 3e). Image quantification analysis demonstrated that the intensity of hypoxia probe in kidneys, lungs and hearts were significantly elevated in A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice compared to E*poR-Cre*$^{+}$ (Supplementary Fig. 3a-c) following hypoxia challenge. Furthermore, histological studies demonstrated that hypoxia-induced severe tissue damage including swollen glomeruli, kidney tubular edema, enlargement of lung airway spaces and damage to pulmonary edema and increased immune cell infiltration in *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice compared to EpoRCre^{+} mice (Fig. 3f). These results show that erythrocyte ADORA2B promotes 2,3-BPG production and O_2 release, thereby protecting hypoxia-induced tissue damage.

AMPK functions downstream of erythrocyte ADORA2B in mice and underlies hypoxia-induced 2,3-BPG production by phosphorylation of 2,3-BPG mutase

Erythrocyte concentrations of 2,3-BPG, an important byproduct of glycolysis, is regulated primarily by the biofunctional enzyme BPG mutase/phosphatase. However, the molecular basis of 2,3-BPG induction in the erythrocytes under hypoxic condition remains unknown. Intriguingly, a previous pulldown study coupled with mass spectral analysis reported that 2,3-BPG mutase binds with AMPK and is likely a substrate of AMPK in erythrocytes [10]. Moreover, early studies showed that ADORA2B signaling can activate AMPK in other cellular systems[11]. Like adenosine, AMPK plays a critical role in multiple cellular functions especially under conditions of energy depletion and

limited O_2 availability[12]. However, whether AMPK functions downstream of ADORA2B as a key enzyme responsible for hypoxia-induced 2,3-BPG production in erythrocytes has not been previously studied. To test this hypothesis, we determined whether hypoxia induced AMPK activation (as judged by phosphorylation at active site (Thr172) of the α subunit) in erythrocytes via ADORA2B signaling *in vivo*. We found that basal AMPKα phosphorylation levels in erythrocytes between A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice and E*poR-Cre*$^{+}$ mice under normoxia were similar (Fig. 4a-b). However, the levels of phosphorylation of AMPKα were significantly induced in the erythrocytes of E*poR-Cre*$^{+}$ mice under 10% O_2 hypoxia, while its induction by hypoxia was significantly attenuated in the erythrocytes of A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ (Fig. 4a-b). These studies demonstrated that hypoxia is capable of inducing AMPK phosphorylation and that erythrocyte ADORA2B is essential for hypoxia-induced AMPK phosphorylation *in vivo*.

Next, we conducted immunoprecipitation experiments to determine if activated AMPK directly phosphorylates 2,3-BPG mutase in erythrocytes. The results of pull down experiments using an antibody that recognizes AMPK substrates showed that p-AMPK specifically phosphorylates 2,3-BPG mutase and that under normoxic conditions level of AMPK phosphorylated 2,3-BPG mutase was similar in A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice and EpoRCre^{+} mice (Fig. 4c-d). Subsequently, we found that hypoxia significantly induced *p*-AMPK-mediated phosphorylation of 2,3-BPG mutase in erythrocytes of E*poR-Cre*$^{+}$ mice and that this phosphorylation was significantly attenuated in A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice (Fig. 4c-d). Additionally, we found that erythrocyte 2,3-BPG levels and

O_2 releasing capacity were significantly reduced in *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice compared to EpoRCre^{+} mice following hypoxia treatment (Fig. 3b-c). Thus, we conclude that ADORA2B-induced AMPK phosphorylation, followed by p-AMPK-mediated phosphorylation of BPG mutase are key steps in the signaling pathway underlying hypoxia-induced 2,3-BPG production and O_2 release from erythrocytes to peripheral tissues in mice.

AMPK activators induce 2,3-BPG production and O_2 release *in vitro* and *in vivo*

Our discovery that erythrocyte AMPK is phosphorylated and subsequently phosphorylates 2,3-BPG mutase in response to ADORA2B signaling suggests that activation of AMPK may be sufficient to induce 2,3-BPG production. To test this possibility we conducted *in vitro* studies to determine if AMPK activation is sufficient to induce 2,3-BPG production in cultured mouse erythrocytes. Initially, we found that two independent AMPK activators, AICAR and metformin, significantly increased *p*-AMPK-mediated phosphorylation of 2,3-BPG mutase (Fig.4e-f). Next, we found that both AMPK activators significantly increased 2,3-BPG production and O_2 release capacity in cultured erythrocytes (Fig.4g-h). Thus, *in vitro* studies demonstrated that AMPK activation is sufficient to induce 2,3-BPG production in mouse erythrocytes.

We extended our *in vitro* studies to further test if metformin, a FDA approved drug, could prevent hypoxia-induced multiple tissue damage in *Cd73*$^{-/-}$ and A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice by stimulating elevated 2,3-BPG and O_2 releasing capacity. We found that metformin pretreatment induced erythrocyte 2,3-BPG levels and O_2 releasing capacity in both *Cd73*$^{-/-}$ and *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice

under hypoxia (Fig.5a-d). Moreover, we found that metformin-mediated elevated 2,3-BPG production and O_2 availability to peripheral organs significantly ameliorated hypoxia-induced multiple tissue damage including lungs, kidneys and hearts in both *Cd73*$^{-/-}$ mice and A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ (Fig.5e). Consistently, image quantification of hypoxia probe staining demonstrated that hypoxia levels were also significantly reduced by metformin treatment in both *Cd73*$^{-/-}$ mice and A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice (Supplementary Fig.4a-c). These studies demonstrate that AMPK functions downstream of erythrocyte ADORA2B adenosine signaling to induce 2,3-BPG production and increase O_2 availability to peripheral tissues. Thus, metformin can override a genetic block to hypoxia-induced adenosine production (i.e., CD73 deficiency) or erythrocyte ADORA2B signaling (erythrocyte-specific ADORA2B deficiency) to stimulate 2,3-BPG production and O_2 release from erythrocytes by the activation of AMPK.

Erythrocyte AMPK phosphorylation is significantly induced at high altitude and correlated to the levels of plasma adenosine, erythrocyte 2,3-BPG and P50 in humans

Our discovery of hypoxia-induced erythrocyte AMPK phosphorylation in mice prompted us to investigate whether increased phosphorylation of erythrocyte AMPK occurs in humans at high altitude. First, we measured erythrocyte AMPK phosphorylation by western blot analysis in human subjects at sea level and at high altitude on ALT1 and ALT16. We found that *p*-AMPK levels of erythrocyte were increased within two hours at high altitude (ALT1) and were further elevated following an extended stay at high altitude (ALT16) (Fig.6a). In addition to western blotting we also

used an ELISA , to accurately quantify *p*-AMPK level of erythrocyte in all human individuals. We found that *p*-AMPK levels were significantly increased on ALT1 and further elevated on ALT16 at high altitude (Fig. 5b). No significant differences in elevated *p*-AMPK levels were observed between male and female subjects in response to high altitude (Supplementary Figure 1i-j). Additionally, levels of AMPK phosphorylation were significantly correlated to the levels of plasma adenosine, soluble CD73 activity, erythrocyte 2,3-BPG and erythrocyte P50 in human subjects (Fig. 6c-f). Finally, we demonstrated that levels of AMPK phosphorylated 2,3-BPG mutase in erythrocytes were significantly increased at ALT1, and further enhanced on ALT16 (Fig. 6g). Overall, we validated our mouse hypoxia findings and provided important human evidence that the phosphorylation of AMPK and BPG mutase are significantly increased at high altitude and that the increase in phosphorylation of these enzymes correlates significantly with increased plasma adenosine and erythrocyte 2,3-BPG levels and O_2 releasing capacity.

Discussion

It has been known for more than four decades that when humans ascend to high altitudes the affinity of Hb to O_2 is decreased to make more O_2 available to hypoxic peripheral tissues[6, 13-15]. It was also recognized early on that the reduced oxygen affinity was due in part to the increase in erythrocyte 2,3-BPG which functioned as a negative allosteric regulator of hemoglobin oxygen affinity[16-18]. The molecular mechanisms accounting for the altitude mediated regulation of erythrocyte 2,3-BPG levels were not understood until we conducted the studies reported here. In a cohort of 19 young healthy volunteers we found that plasma adenosine levels and soluble CD73 activity were elevated within two hours of arrival at 5260 m and increased further upon prolonged stay at that altitude. Our studies showed that elevated adenosine levels were proportional to soluble CD73 activity and that the two were highly correlated to increased 2,3-BPG levels and O_2 releasing capacity of erythrocytes in humans at high altitude. We reported similar finding with mice when placed under experimental conditions that mimic the 10% oxygen levels present at 5260 m. Using the mouse model we showed that hypoxia-induced soluble CD73 accounts for the increase in circulating adenosine and that increased plasma adenosine signaling via erythrocyte ADORA2B induces 2,3-BPG production, triggers O_2 release and prevents hypoxia-mediated tissue injury. We determined that AMPK functions downstream of ADORA2B and is responsible for hypoxia-induced 2,3-BPG production and subsequent O_2 release from erythrocytes. The mechanistic studies showing hypoxia induced phosphorylation of erythrocyte AMPK in mice were followed by translational studies showing altitude dependent phosphorylation of erythrocyte AMPK in human subjects.

Overall, both human and mouse studies reported here provide strong evidence that CD73-dependent elevation of plasma adenosine signaling via ADORA2B on erythrocyte has a beneficial role by preventing altitude hypoxia-mediated tissue damage by inducing 2,3-BPG production and triggering O_2 release in a AMPK-dependent manner (Fig.6i). Thus, our findings reveal novel therapeutic targets for high altitude sickness (HAS) and hypoxia-induced tissue damage and provide a strong foundation for future clinical trials.

The physiology of high altitude adaptation to hypoxia has been extensively studied, however, the cellular and molecular basis underlying high altitude acclimatization is poorly understood. It has been long speculated that one of the major mechanisms of high altitude adaptation is to induce erythrocyte O_2 releasing capacity to counteract hypoxia-induced tissue damage[6]. To function effectively in O_2 uptake, transport and delivery, erythrocytes rely on sophisticated regulation of hemoglobin (Hb)-O_2 affinity by endogenous allosteric modulators. One of the best known allosteric modulators is 2,3-BPG, a metabolic byproduct of glycolysis synthesized primarily in erythrocytes for the purpose of regulating Hb-O_2 affinity. Earlier studies demonstrated that erythrocyte 2,3-BPG levels are elevated at high altitude and its elevation is significantly correlated to O_2 releasing capacity[6]. However, the molecular basis underlying its induction remained unidentified and its role in altitude hypoxia-induced tissue damage was unclear until now. Although adenosine is known to be induced under hypoxia, no prior study has reported that circulating adenosine levels are increased at high altitude and no role of elevated adenosine in high altitude adaptation has been recognized prior

to our study with young healthy human subjects. We show that plasma adenosine levels are increased within two hours of arrival at high altitude and increased further upon prolonged stay at high altitude. Consistent with the altitude-dependent increase in plasma adenosine, we also found that soluble CD73 activity was also significantly induced by high altitude and that the increase in soluble CD73 activity was proportional to increased plasma adenosine levels. Moreover, we confirmed early studies showing that erythrocyte 2,3-BPG and O_2 releasing capacity are significantly elevated in humans at high altitude. The elevated plasma adenosine levels and soluble CD73 activity were proportional to increased erythrocyte 2,3-BPG levels and O_2 releasing capacity. Of note, gender had no effects on high altitude-induced increased plasma adenosine, soluble CD73 activity, 2,3-BPG levels and O2 releasing capacity.

In response to hypoxia, extracellular adenosine is generated from extracellular AMP by ecto-5'-nucleotidase (CD73). Accumulated excess circulating adenosine stimulates specific adenosine receptors and further activates downstream signaling cascades to regulate multiple cellular and systemic functions under physiological and pathological settings[19]. Acutely elevated plasma adenosine is brief and considered to be largely beneficial [20-24]. For example, in response to tissue hypoxia elevated adenosine signaling induces vasodilation, decreased heart rate an inflammatory response and increased wound healing. In contrast, prolonged persistently elevated plasma adenosine is widely considered to be detrimental by inducing fibroblast cell proliferation, activating mast cells

and macrophage cells and increasing vascular smooth cell proliferation and thereby contributes to multiple chronic diseases including COPD, asthma, chronic kidney disease, sickle cell disease and priapism. Although adenosine signaling regulates numerous cellular and tissue functions by engaging its membrane receptors[8, 25], nothing is known about the physiological function of adenosine and ADORA2B signaling in normal erythrocytes. Our human studies presented here support the hypothesis that elevated plasma adenosine is dependent on elevated CD73 and that elevated adenosine has a previously unrecognized role in high altitude adaption by regulating 2,3-BPG production and O_2 releasing capacity in erythrocytes.

In an effort to identify the signaling pathways underlying the altitude dependent induction of erythrocyte 2,3-BPG we turned to an experimental model in which mice are maintained in an environment of 10% oxygen, resembling that found at 5260 m where our experimental human subjects were examined. Similar to humans at high altitude, we found that soluble CD73 activity, circulating adenosine levels, erythrocyte 2,3-BPG levels and O_2 releasing capacity were significantly elevated in the wild type mice maintained for 1 week at 10% O_2 hypoxia. However, genetic deletion of CD73 significantly reduced hypoxia-induced plasma adenosine, erythrocyte 2,3-BPG levels and O_2 release capacity in the mice. The decreased O_2 availability to peripheral tissues resulted in severely hypoxic organs and multiple tissue injury. Consistent with our findings, early studies showed that genetic deletion of CD73 leads to vascular leakage and increased immune cell

infiltration in the lungs under hypoxic condition[26, 27]. However, the functional role of CD73-dependent elevated circulating adenosine in the induction of 2,3-BPG levels and O_2 release capacity from normal erythrocytes to protect hypoxic tissue damage has not been previously recognized prior to our studies. To address the *in vivo* significance of erythrocyte ADOAR2B signaling in hypoxia-induced tissue damage we generated mice with specific ablation of ADORA2B on the erythrocytes. These newly developed erythrocyte specific ADORA2B knockouts allowed us to discover that erythrocyte ADORA2B is essential for elevated plasma adenosine induced-2,3-BPG production and O_2 releasing capacity from erythrocytes. The ablation of erythrocyte ADORA2B in mice results in more hypoxia-induced tissue and organ damage, similar to CD73-deficient mice. Taken together, we have shown that elevated plasma adenosine signaling via erythrocyte ADORA2B plays an important role in preventing hypoxia-induced tissue damage by inducing erythrocyte 2,3-BPG levels and triggering O_2 release to local tissues. Thus, our findings have revealed a novel role of erythrocyte ADORA2B signaling to protect hypoxia-induced tissue damage by inducing 2,3-BPG production and promoting O_2 release.

AMPK is a well-known metabolic stress-sensing kinase, which plays an essential role in regulating cellular energy metabolism. [28, 29] In erythrocytes, AMPK plays critical roles in regulating oxidative stress and maintaining the integrity and life span of the cell[30-33]. A Previous study used a proteomic approach to identify BPG-mutase as an AMPK target in RBC[10]. However, a role for AMPK in 2,3-BPG induction under high altitude or hypoxic conditions in normal erythrocytes has

not been previously investigated. Our current work revealed that AMPK functioning downstream of ADOARA2B directly phosphorylates 2,3-BPG-mutase resulting in increased production of 2,3-BPG, thereby promoting O_2 release. Moreover, we demonstrated that two independent AMPK activators induce phosphorylation of 2,3-BPG mutase, 2,3-BPG production and O_2 release from cultured mouse erythrocytes. Because of the importance of AMPK in the induction of 2,3-BPG production in erythrocytes, we conducted preclinical studies and found that pretreatment with metformin, a FDA-approved drug that has been used safely to treat patients with type 2 diabetes since 1957[34], prevented hypoxia-induced tissue damage in both CD73-deficient mice and erythrocyte specific ADORA2B-deficient mice by inducing erythrocyte 2,3-BPG levels and O_2 availability to local tissue. Extending mouse studies, we validated that AMPK activity is significantly elevated in the erythrocytes of humans under high altitude. Significantly, AMPK phosphorylation also correlated to the changes in circulating adenosine levels, soluble CD73 activity, erythrocyte 2,3-BPG levels and O_2 releasing capacity in human subjects as a function of high altitude. Thus, both human translational studies and preclinical studies in mice provide a strong foundation for future clinical trials in humans to test the ability of metformin to increase O_2 availability in humans. Moreover, transition to future clinical trials to treat or prevent HAS or any hypoxia-induced diseases should be rapid since metformin is a FDA approved drug.

In conclusion, our discovery that increased circulating adenosine promotes O_2 release by activating ADORA2B on erythrocytes is especially innovative since the functional role of elevated extracellular adenosine signaling in O_2 release from Hb in erythrocyte had not been previously recognized.

Moreover, our finding that AMPK, as a key energy sensor functioning downstream of ADORA2B in erythrocytes, is involved in triggering O_2 release by directly inducing 2,3-BPG production is also novel. Finally, our findings are clinically significant since human translational studies showed that AMPK activity is induced by altitude hypoxia and our preclinical studies demonstrated that metformin treatment improves oxygen delivery to prevent tissue damage in conditions involving systemic hypoxia. Thus, our current studies have added significant new insight to the molecular basis underlying high altitude hypoxia adaptation and thereby have opened up novel therapeutic possibilities for prevention and treatment of HAS and other hypoxia-related diseases.

Materials and Methods

Human Subjects

This study was conducted as part of the Altitude0mics project examining the integrative physiology of human responses to hypoxia (citations). Unique data presented here regarding the adenosine-erythrocyte response during adaptation to hypoxia. In brief, all procedures conformed to the Declaration of Helsinki and were approved by the Universities of Colorado and Oregon Institutional Review Boards and the US Department of Defense Human Research Protection 0ffice. After written informed consent, ___ (___ male) recreationally active sea-level habitants participated in the adenosine study (mean±SD age, 21±1 years; stature, 1.78±0.10 m; body mass, 69±11 kg; maximum 02 uptake [Vo2max, 6.4±0.2 mL kg1 min1 [participant IDs: fill in from Hong's latest list). The participants were non-smokers, free from cardiorespiratory disease, born and raised at <1500 m, and had not travelled to elevations >1000 m in the 3 months prior to investigation.

Each subject was studied near sea level (SL) (130 m, average PB=749 mmHg, Figure 1) and on the first and sixteenth days Mt Chacaltaya, Bolivia (5260 m; average PB=406 mmHg; ALT1, ALT16). Baseline studies at SL were conducted over a two-week period in Eugene, OR, USA. Approximately one month after the SL studies, subjects traveled to Bolivia in pairs on successive days. Upon arrival at El Alto (4050m) after an overnight flight, subjects immediately descended to Coroico, Bolivia (1525 m; PB=639 mmHg). Subjects rested for 48 hrs in Coroico to limit the effects of jet lag and were then driven over three hrs to 5260 m. To provide an acute change in inspired PO2 from 1525 m to 5260 m, subjects breathed supplemental oxygen (2 L/min, nasal cannula or mask) during the drive. On arrival at 5260 m, the first subject immediately began the experimental protocol described below.

Mouse Subjects

8 to 10-week-old C57BL/6 wild-type (WT) mice were purchased from Harlan Laboratories

(Indianapolis, IN). Ecto-5'-nucleotidase (CD73)–deficient mice and A2B adenosine receptor (ADORA2B)–deficient mice congenic on a C57BL/6 background were generated and genotyped as described before[36]. A novel line of mice with erythrocyte specific deletion of *Adora2b* was generated by crossing mice homozygous for a floxed *Adora2b* allele with mice expressing Cre recombinase under the control of erythropoietin receptor (EpoR) gene regulatory elements. All protocols involving animal studies were reviewed and approved by the Institutional Animal Welfare Committee of the University of Texas Houston Health Science Center.

Blood collection and hematological analysis

Human and mouse blood were collected and stored as described before respectively[9, 37]. Briefly, approximately 4 ml of human blood was collected with EDTA as an anti-coagulant and complete blood count (CBC) and 1 ml of blood was aliquoted to tubes containing 10 μM dipyridamole inhibitor of equilibrative nucleoside transporters) and 10 μM deoxycoformycin (inhibitor of adenosine deaminase) for plasma adenosine assay. 4 ml of blood was collected with heparin as an anti-coagulant for 2,3-bisphosphoglycerate (2,3-BPG) measurement followed the protocol. Mouse blood was collected similar to human blood as described above, but at smaller volumes. Human blood was frozen at -80C after collection and during shipment from the field until assay.

Measurement of soluble CD73 activity in mouse

Circulating CD73 enzyme activity was measured by quantifying the conversion of etheno-AMP (E-AMP) to ethenoadenosine (E-ADO) as described previously([36, 38]). Briefly, frozen plasma were thawed and used to measure CD73-specific activity. First, 20μl plasma was preincubated at room temperature with 200 nM deoxycoformycin in 0.1 M HEPES (pH 7.4), with 50 μM MgCl, with or without α,β-methylene ADP (APCP, Sigma-Aldrich, a specific inhibitor of CD73). Next, samples were incubated at 37°C for 30 min in the presence of 100 μM AMP. AMP hydrolytic activity (AMPase) was measured by determining adenosine concentrations with reversed phase HPLC as describe before. Absorbance was measured at 260 nm, and ultraviolet absorption spectra were obtained at chromatographic peaks. CD73 activity was expressed as relative E-AMP conversion .

Measurement of soluble CD73 activity in human

Circulating CD73 enzyme activity in human was measured by quantifying the conversion of [^{3}H]-labeled AMP to [^{3}H]-labeled adenosine as described previously([39, 40]) Briefly, soluble CD73 activity was measured after incubation of plasma with 300 uM [^{3}H]AMP(Quotient Bioresearch; GE Healthcare, Rushden, UK) for 60min at 37°C. Mixture then was applied onto Alugram SIL G/UV254 sheets (Macherey-Nagel, Germany). Radiolabelled substrates and their products were separated by thin layer chromatography (TLC) and quantified by scintillation β -counting (Yegutkin et al. 2001).

Adenosine measurement

Adenosine concentration in plasma was measured by HPLC as previously described with modification[9, 36]. In brief, 500 µl plasma was mixed with 500 µl 0.6 M cold perchloric acid on ice, subsequently vortexed, and sonicated for 10 seconds. The homogenate was centrifuged at 20,000 x g for 10 min at 4 ^{0}C. The supernatant (568 µl) was transferred to a new tube and neutralized with 40.9 µl 3 M $KHCO_3$/3.6 N KOH. Additionally, phenol red (2 µl of 0.2 mg ml^{-1}) was added as indicator. The sample was subsequently acidified with 5.7 µl of 1.8 M ammonium dihydrogen phosphate (pH 5.1) and 13.2 µl phosphoric acid (30%). Then, the sample was centrifuged at 20,000 x g for 5 min and the supernatant was transferred to a new tube and ready for HPLC analysis as described previously. Adenosine content was normalized to volume and expressed as a concentration.

Isolation of total erythrocytes and treatment of mouse erythrocytes *in vitro*.

Blood was collected with heparin as an anti-coagulant and centrifuged at 240 x g for 10 min at room temperature, followed by aspiration of plasma and white interface[9, 37]. Packed red blood cells (RBCs) were washed 3 times with culture media (F-10 Ham's with 1% penicillin/streptomycin, 10% fetal bovine serum (FBS), and re-suspended to 4% hematocrit (HCT). One ml of RBCs was added to each well of a 12-well plate. Mouse RBCs were treated with AICAR (TOCRIS, USA) and metformin(Sigma, USA) at 1mM for different time points (from 1 to 4h hr) under normoxic conditions[31]. At the end of experiments, 2,3-BPG levels were measured, the hemoglobin-O_2 binding affinity were analyzed and calculated as P50 by Hemox analyzer.

Mouse organ isolation and histological analysis.

Mice were anesthetized and body weight was determinedOrgan was fixed with 4% paraformaldehyde in PBS overnight at 4 °C. Fixed tissues were rinsed in PBS, dehydrated through graded ethanol washes, and embedded in paraffin. 4 µm sections were collected on slides for **immunohistochemistry** or haematoxylin and eosin (H&E) staining.

Hypoxia probe immunohistochemistry in the kidney, lung and heart with quantification

Tissue hypoxia level were assessed by Hypoxia probe immunohistochemistry as described before[38]. Briefly, animals were administered Hypoxyprobe (Hypoxiaprobe, Inc.) via intraperitoneal injection (60 mg/kg body weight). One hour after injection, tissues including heart, lungs and kidneys were harvested, fixed overnight in 4% buffered formalin, and embedded in paraffin. According to the manufacturer's instructions (Hypoxyprobe-1 Omni-Kit), sections of 4µm were cut and mounted on glass slides, deparaffinized through serial baths in xylene and rehydrated in a graded series of alcohol and distilled water. Endogenous peroxidase activity was quenched by 10 min of incubation in a 3% hydrogen peroxide/methanol buffer. Antigen retrieval was enhanced by autoclaving slides in sodium citrate buffer (pH 6.0) at 95°C for 15 min. Next, endogenous avidin and biotin blocking was performed with a Biotin Blocking System (Dako). The slides were then incubated with rabbit anti-PAb2627AP in a humidified chamber at 4°C overnight. After the primary

antibody incubation, anti-rabbit IgG ABC staining system kit (VEACTASTAIN ABS-AP, VECTOR LAB) was used according to the protocol. Slides were subsequently stained with alkaline phosphatase substrate kit (VEACTASTAIN ABS-AP, VECTOR LAB) and counterstained with hematoxylin. Quantification of the immunohistochemical staining was performed using the Image-Pro Plus software (Media Cybernetics, Bethesda, MD). The density of the red staining was measured. The average densities of 20 areas per samples were determined and the SEM is indicated. n=6 for each group.

Immunoprecipitation

Erythrocyte pellets were lysed in lysis buffer (1XTBS, 1% NP-40, 5 mM EDTA), protease inhibitor cocktail (Sigma-Aldrich, MO), and phosphatase inhibitor cocktail (Sigma-Aldrich, MO). The total protein concentration was measured with a Protein Assay Kit (Bio-Rad). Cell lysates were precleared with 50 µl Protein A/G Sepharose High Performance beads (GE Healthcare Life Sciences), and then incubated overnight at 4^oC with 50 µl antibody-bound beads prepared according to manufacturer's instructions and rocked gently. After immunoprecipitation, the beads were washed 4 times with 1XTBS, and boiled with 2X Laemmli buffer for Western blot analysis.

Western blot analysis

Protein in human and mice erythrocytes was analyzed by western blotting. Frozen erythrocyte pellets were used for protein extraction. Proteins were run on 5-20% SDS-PAGE gels and transferred to nitrocellulose membrane. Membranes were incubated with anti-BPG mutase antibody (Santa Cruz.), anti-AMPKa and anti-pAMPKa (Cell Signaling.) respectively as primary antibody.

Statistics.

All data are expressed as the mean ± SEM. Data were analyzed for statistical significance using GraphPad Prism 5 software (GraphPad Software). Two-tailed Student' s *t* tests (paired or unpaired as appropriate) were applied in 2-group analysis. Differences between the means of multiple groups were compared by one-way analysis of variance, followed by a Turkey's multiple comparisons test. P value of less than 0.05 was considered significant. The relationship between two variables X and Y were analyzed by Pearson product-moment correlation coefficient method. $P<0.05$ (two-sided) was considered statistically significant. The linear correlation (dependence) is described as R square.

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Figure Legends

Figure 1. Altitude hypoxia-induced plasma adenosine levels and soluble CD73 activity correlate to increased erythrocyte 2,3-BPG levels and O_2 releasing capacity in humans. (**a-b**) Plasma adenosine levels and soluble CD73 activity were induced by high altitude. Plasma adenosine levels (**a**) and soluble CD73 activity (**b**) of normal individuals at sea level (SL) and at high altitude on day 1 (ALT1) and day 16 (ALT16). Data are expressed as mean ± SEM; *P<0.05 vs SL; **P<0.05 vs ALT1. n=19. (**c**) Plasma adenosine levels correlated significantly with plasma CD73 activity of normal individuals at SL, ALT1 and ALT16 (R^2 =0.3664, p<0.0001). (**d-e**) Erythrocyte 2,3-BPG concentration and P50 were induced by high altitude. Erythrocyte 2,3-BPG levels (**d**) and P50 (**e**) at sea level and at high altitude on day 1 and day 16. Data are expressed as mean ± SEM; *P<0.05 vs SL; **P<0.05 vs ALT1. n=19. (**f-g**) Plasma adenosine levels correlated significantly to erythrocyte 2,3-BPG levels (R^2 =0.3664, p<0.0001) and P50 (R^2 =0.1915, p<0.01) at sea levels and high altitude on day 16, respectively.

Figure 2. CD73-mediated elevated extracellular adenosine is essential for increased erythrocyte 2,3-BPG and O_2 releasing capacity to protect hypoxia-induced tissue damage in mice. WT mice and *Cd73*$^{-/-}$ mice were exposed to normoxia or hypoxia (10% O_2, 90% N_2) for 1 wk. (**a-b**) CD73 is essential for hypoxia-induced plasma adenosine in mice. Soluble CD73 activity (**a**) and plasma adenosine (**b**) in WT mice and *Cd73*$^{-/-}$ mice under normoxia or hypoxia conditions. (**c-d**) CD73 is

required for hypoxia-induced erythrocyte 2,3-BPG levels and P50. Erythrocyte 2,3-BPG levels and P50 in WT mice and *Cd73*$^{-/-}$ mice under normoxia or hypoxia conditions. Data are expressed as mean ± SEM; *P<0.05 vs WT under normoxia condition; **P<0.05 vs WT under hypoxic condition. (n=8-10 mice per group). (**e-f**) Correlation of plasma adenosine to plasma CD73 activity (e, R^2 =0.6607, p<0.0005), erythrocyte 2,3-BPG (f, R^2 =0.6471, p<0.0001) under nomoxia or hypoxia conditions in WT mice. (**g**) Immunohistochemical analysis of tissue hypoxia and histological changes in kidney, lung and heart of WT mice and *Cd73*$^{-/-}$ mice under normoxia or hypoxia conditions (n=8-10 mice per group; Scale bars, =400μm).

Figure 3. Erythrocyte ADORA2B contributes to hypoxia-induced 2,3-BPG production and O_2 release capacity and protects mice from hypoxia-induced tissue damage. EpoRCre^{+} mice and *Adora2b*$^{f/f}$/*EpoR-Cre*$^{+}$ mice were exposed to normoxia or hypoxia (10% O_2, 90% N_2) for 1 wk. (**a-c**) ADORA2B on erythrocytes contributes to hypoxia-induced 2,3-BPG production and P50. Plasma adenosine level (a), erythrocyte 2,3-BPG (b) and P50 (c) of E*poR-Cre*$^{+}$ mice and *Adora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice under normoxia or hypoxia condition. Data are expressed as mean ± SEM; *P<0.05 vs E*poR-Cre*$^{+}$ mice under normoxia condition; $^{\#}$P<0.05 vs E*poR-Cre*$^{+}$ mice under hypoxic condition. (n=8-10 per group). (**d**) Correlation of plasma adenosine to erythrocyte 2,3-BPG levels (d, R^2 =0.6489, p<0.0005) under nomoxia or hypoxia conditions in E*poR-Cre*$^{+}$ mice. (**f**) Immunohistochemical analysis of tissue hypoxia and histological changes in kidney, lung and heart

of *EpoR-Cre*$^{+}$ mice and *Adora2b*$^{f/f}$/*EpoR-Cre*$^{+}$ mice under normoxia or hypoxic conditions. (n=8-10 mice per group; scale bars=400μm)

Figure 4. AMPKα functions downstream of ADOAR2B underlying hypoxia-induced 2,3-BPG production by phosphorylation of-2,3-BPG mutase in mice. (**a-b)** Erythrocyte ADORA2B is essential for hypoxia-induced phosphorylation of AMPKα (p-AMPKα) *in vivo*. (**a**) Representative western blot of phosphorylation of AMPKα in the erythrocytes of *EpoR-Cre*$^{+}$ mice and *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice under normoxia or hypoxia (10% O_2, 90% N_2) for 1 week. (**b**) Image quantification analysis showing that p-AMPKα protein levels were significantly induced in the erythrocytes of the control *EpoR-Cre*$^{+}$ mice under hypoxia and hypoxia-induced p-AMPK level was significantly attenuated in the erythrocytes of *Adora2b*$^{f/f}$/EpoR-Cre^{+} mice. Data are expressed as mean ± SEM; *P<0.05 for hypoxia exposed *EpoR-Cre* mice vs *EpoR-Cre* mice under nomoxia condition; $^{\#}$P<0.05 for hypoxia-exposed *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice vs hypoxia-exposed *Epo-Cre* mice. (n=3). (**c-d**) Erythrocyte ADORA2B is critical for hypoxia-induced AMPKα (p-AMPKα)-mediated phosphorylation of 2,3-BPG mutase *in vivo.* (**c**) Representative western blot analysis of phosphorylated 2,3-BPG mutase levels in the erythrocyte lysates immunoprecipated by an antibody specific for phosphorylated AMPK substrates in *EpoR-Cre*$^{+}$ mice and *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice under normoxia or hypoxia conditions. (**d**) Image quantification analysis showing that levels of interaction of P-AMPKα with 2,3-BPG mutase were significantly induced by hypoxia in the erythrocytes of the control *Epo-Cre* mice, while hypoxia-induced interaction of P-AMPKα with 2,3-

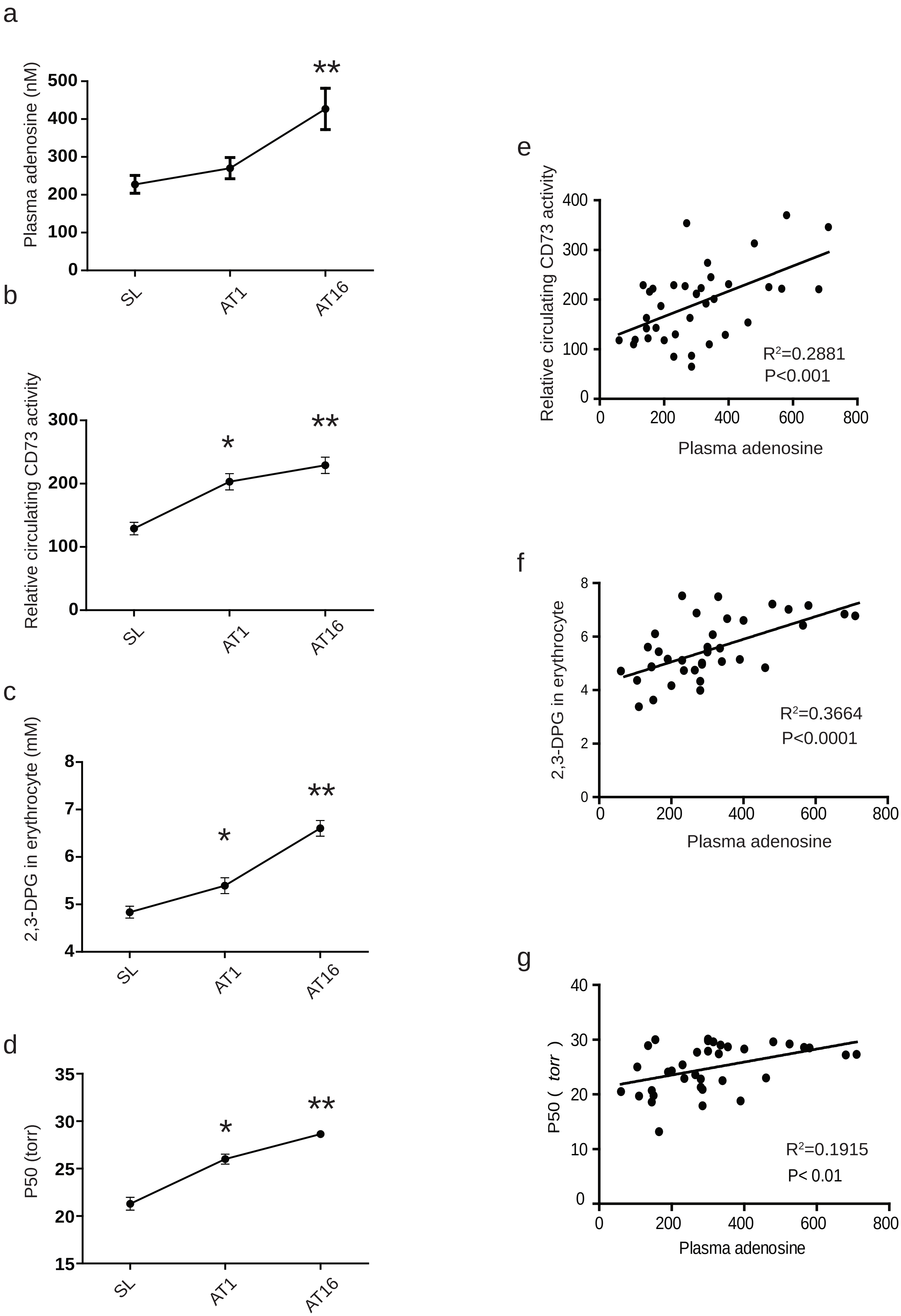
BPG mutase was significantly reduced in the erythrocytes of *Adora2b$^{f/f}$/EpoR-Cre^{+}* mice. Data are expressed as mean ± SEM; *P<0.05 for hypoxia exposed *EpoR-Cre^{+}* mice vs *EpoR-Cre^{+}* mice under nomoxia condition; $^{\#}$P<0.05 for hypoxia-exposed *Adora2b$^{f/f}$/EpoR-Cre^{+}* mice vs hypoxia-exposed *EpoR-Cre^{+}* mice. n=3 (**e**) Representative western blot analysis of phosphorylated 2,3-BPG mutase levels in lysates of DMSO, Metformin or AICAR-treated primary mouse erythrocytes immunoprecipated by an antibody specific for phosphorylated AMPK substrates. (**f**) Image quantification analysis showing that levels of p-AMPK-mediated phosphorylation of 2,3-BPG mutase were significantly induced by metformin or AICAR treatment in the cultured primary mouse erythrocytes (n=6-8). (**g-h**) AMPK agonists including Metformin and AICAR directly induced 2,3-BPG concentrations (**g**) and P50 (**h**) in cultured primary erythrocytes isolated from wild type mice. Data are expressed as mean ± SEM; *P<0.05 for Metformin or AICAR treated mouse erythrocytes vs DMSO-treated cells (n=6-8 mice per group)

Figure 5. Metformin therapy promotes erythrocyte 2,3-BPG production and O_2 release and rescues hypoxia-induced tissue damage in *Cd73$^{-/-}$* mice and *Adora2b$^{f/f}$/EpoR-Cre^{+}* mice. WT mice, *CD73$^{-/-}$* mice, *EpoR-Cre^{+}* mice and *Adora2b$^{f/f}$/EpoR-Cre^{+}* mice were exposed to hypoxia (8% O_2, 92% N_2) for 72 hours. (**a-b**) Metformin treatment stimulated hypoxia-induced erythrocyte 2,3-BPG production (**a**) and P50 (**b**) in *Cd73$^{-/-}$* mice to similar levels as seen in WT mice. Data are expressed as mean ± SEM; *P<0.05 for metformin treated *Cd73$^{-/-}$* mice vs saline-treated *Cd73$^{-/-}$* mice. (n=6-8 mice per group) (**c-d**) Metformin treatment stimulated hypoxia-induced erythrocyte 2,3-BPG production (**c**) and P50 (**d**) in *Adora2b$^{f/f}$/EpoR-Cre^{+}* mice to the similar levels as *EpoR-Cre^{+}* mice.

Data are expressed as mean ± SEM; * P<0.05 for metformin treated *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice vs saline-treated *Adora2b*$^{f/f}$*/EpoR-Cre*$^{+}$ mice. (n=6-8 mice per group) (**e**) Metformin treatment prevented hypoxia-induced multiple tissue damage. Immunohistochemical analysis of tissue hypoxia and histological changes in kidney, lung and heart of saline or metformin-treated *Cd73*$^{-/-}$ and *Adora2b*$^{f/f}$/*EpoR-Cre+* mice.

Figure 6. Erythrocyte AMPKα and BPG mutase phosphorylation is significantly induced at high altitude and correlated to the levels of plasma adenosine and erythrocyte 2,3-BPG and P50 in humans. (**a**-**b**) High altitude induced phosphorylation of AMPKα (p-AMPKα) in the erythrocytes of normal individuals. (**a**) Representative western blot of phosphorylation of AMPKα in the erythrocytes of humans at sea level and high altitude on day 1 and day 16. Lower panel: Image quantification analysis showing that p-AMPKα protein levels were significantly induced in the erythrocytes of human under high altitude on day 1 and further increased on day 16. (**b**) ELISA accurately quantified erythrocyte p-AMPKα in humans. Data are expressed as mean ± SEM; *P<0.05 for erythrocyte p-AMPKα at high altitude on day 1 vs sea level; $^{\#}$P<0.05 erythrocyte p-AMPKα at high altitude on day 1 vs day 16. n=19. (**c-f**) Elevated erythrocyte p-AMPKα levels were significantly correlated to plasma adenosine (c, R^2 = 0.2228, p<0.005), soluble CD73 activity (d, R^2 =0.3216, p<0.0005), 2,3-BPG (e, R^2 =0.4535, p< 0.0001) and P50 (f, R^2 =0.5012, p< 0.0001) of normal individuals at sea levels and at high altitude day 16 (ALT16). (**g-h**) High altitude induced p-AMPKα-mediated phosphorylation of erythrocyte 2,3-BPG mutase in the erythrocytes of normal

humans. (**g**) Representative western blot analysis of phosphorylated 2,3-BPG mutase levels in the erythrocyte lysates immunoprecipated by an antibody specifically against phosphorylated AMPKα substrates at sea levels and high altitude on day 1 and day 16. (**h**) Image quantification analysis showing that levels of P-AMPKα-mediated phosphorylation of 2,3-BPG mutase were significantly induced on day 1 and further elevated on day 16 compared to sea level. Data are expressed as mean ± SEM; *P<0.05 for erythrocyte p-AMPKa at high altitude on day 1 vs sea level; $^{\#}$P<0.05 erythrocyte p-AMPKα at high altitude on day 1 vs day 16. (n=3 per group) (**i**) Working model: CD73 is essential for altitude hypoxia-induced circulating adenosine production. Elevated circulating adenosine protects against hypoxia-induced tissue damage by activating ADORA2B on erythrocytes to induce 2,3-BPG production and trigger O_2 release to peripheral ischemic tissues. AMPKα is a key enzyme functioning downstream of ADORA2B to promote 2,3-BPG induction and O_2 release from erythrocytes. Overall, CD73-dependent elevation of circulating adenosine is beneficial to protect hypoxia-induced tissue damage by signaling via erythrocyte ADOAR2B to promote 2,3-BPG production and O_2 release from erythrocytes to peripheral tissue in a AMPK-dependent manner. Thus, enhancing the CD73-ADORA2B-AMPK signaling pathway is a promising therapeutic strategy to treat or prevent HAS and hypoxia-induced tissue damage.


a
Plasma adenosine (nM)
500
400
300
200
100
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SL
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Relative circulating CD73 activity
300
200
100
0
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SL
AT1
AT16
c
2,3-DPG in erythrocyte (mM)
8
7
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SL
AT1
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P50 (torr)
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SL
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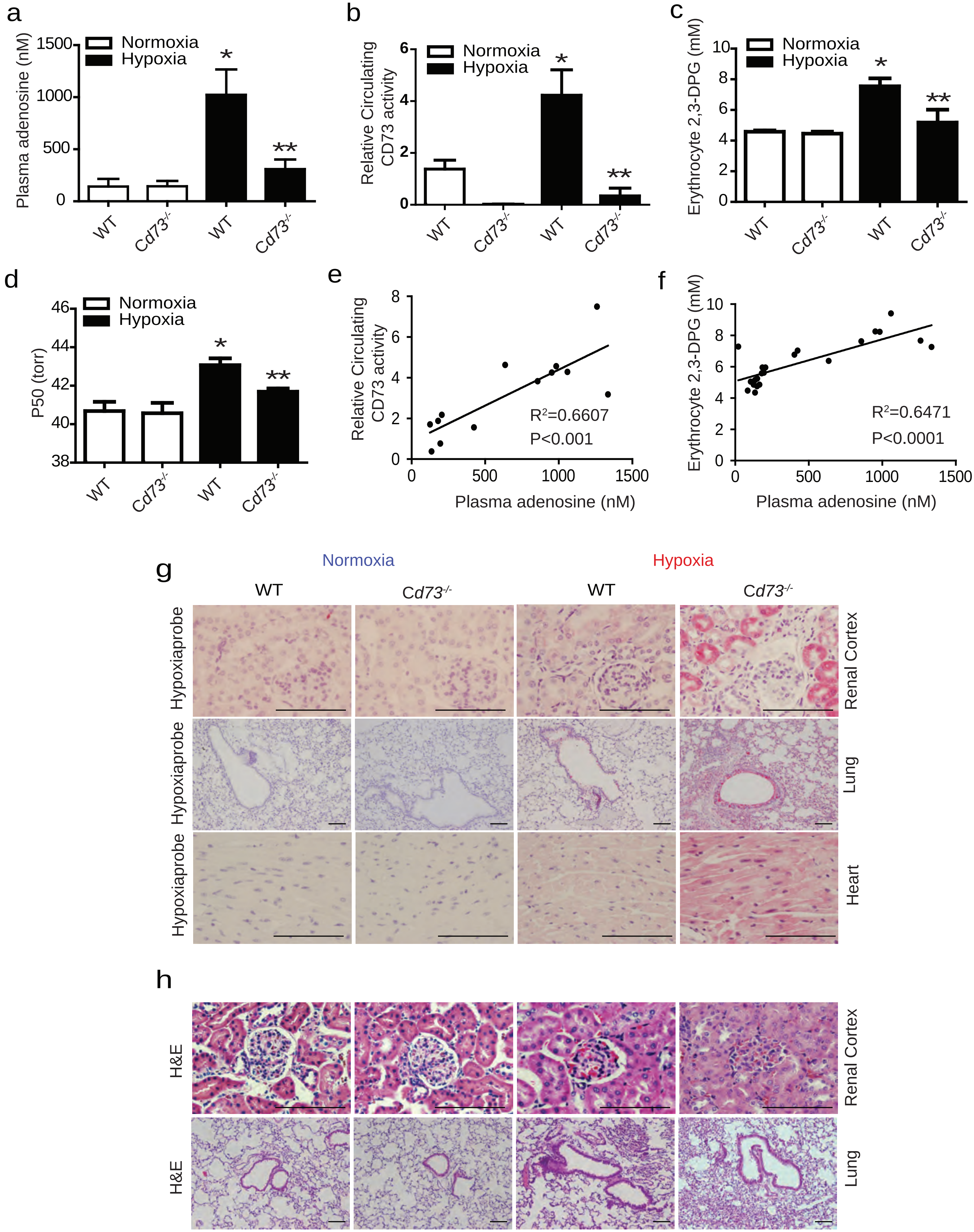
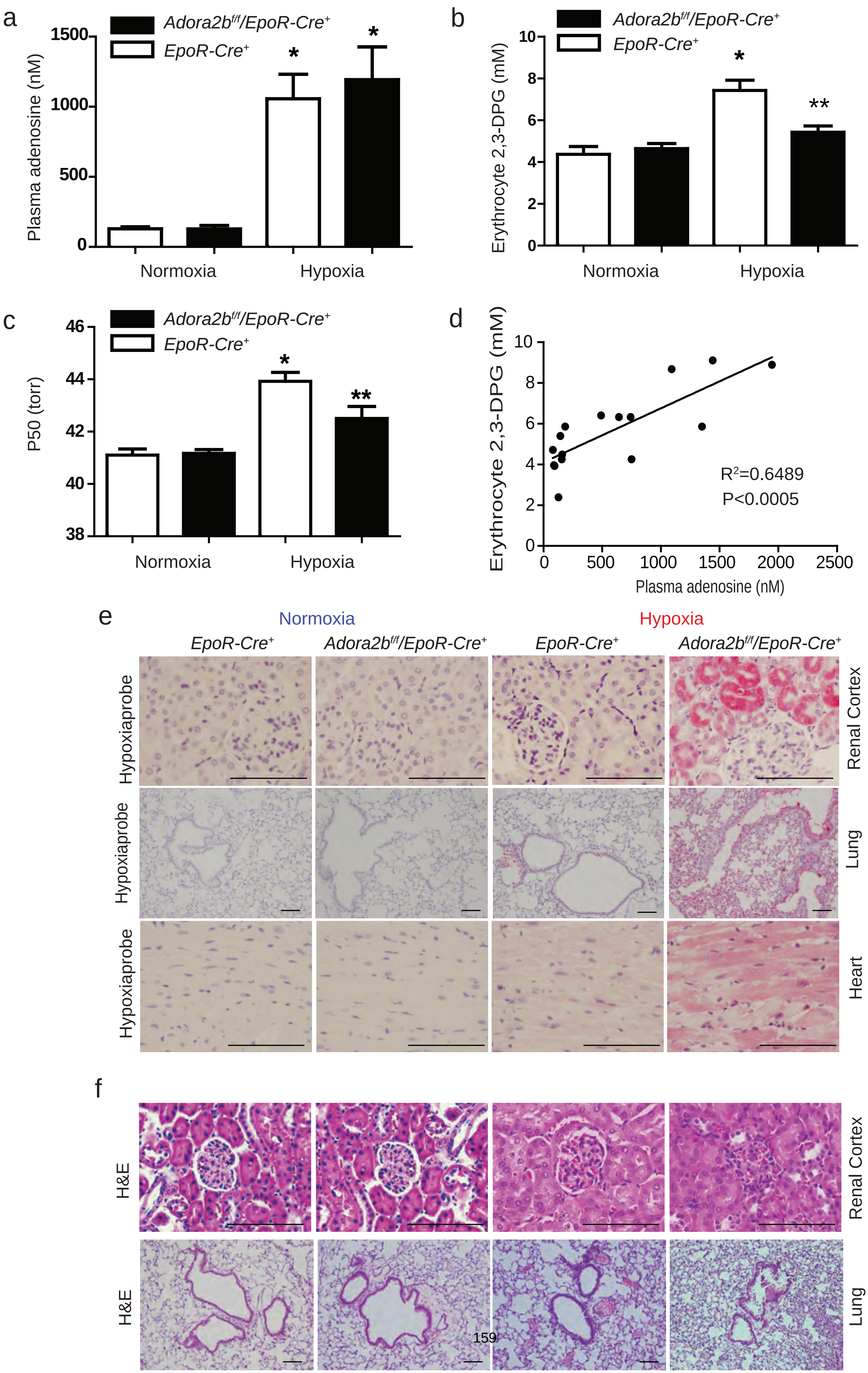
e
Relative circulating CD73 activity
400
300
200
100
0
0
200
400
600
800
Plasma adenosine
R²=0.2881
P<0.001
f
2,3-DPG in erythrocyte
8
6
4
2
0
0
200
400
600
800
Plasma adenosine
R²=0.3664
P<0.0001
g
P50 (torr)
40
30
20
10
0
0
200
400
600
800
Plasma adenosine
R²=0.1915
P< 0.01

Fig. 1



a
Plasma adenosine (nM)
1500
1000
500
0
Normoxia
Hypoxia
*
**
WT
Cd73-/-
WT
Cd73-/-
b
Relative Circulating CD73 activity
6
4
2
0
Normoxia
Hypoxia
*
**
WT
Cd73-/-
WT
Cd73-/-
c
Erythrocyte 2,3-DPG (mM)
10
8
6
4
2
0
Normoxia
Hypoxia
*
**
WT
Cd73-/-
WT
Cd73-/-
d
P50 (torr)
46
44
42
40
38
Normoxia
Hypoxia
*
**
WT
Cd73-/-
WT
Cd73-/-
e
Relative Circulating CD73 activity
R2=0.6607
P<0.001
Plasma adenosine (nM)
0
500
1000
1500
f
Erythrocyte 2,3-DPG (mM)
R2=0.6471
P<0.0001
Plasma adenosine (nM)
g
Normoxia
Hypoxia
WT
Cd73-/-
WT
Cd73-/-
Hypoxiaprobe
Renal Cortex
Lung
Heart
h
H&E
Renal Cortex
Lung

Fig. 2



a
Adora2bf/f/EpoR-Cre+
EpoR-Cre+
Plasma adenosine (nM)
1500
1000
500
0
Normoxia
Hypoxia
b
Erythrocyte 2,3-DPG (mM)
10
8
6
4
2
0
c
P50 (torr)
46
44
42
40
38
d
Erythrocyte 2,3-DPG (mM)
R2=0.6489
P<0.0005
Plasma adenosine (nM)
0
500
1000
1500
2000
2500
e
Normoxia
Hypoxia
EpoR-Cre+
Adora2bf/f/EpoR-Cre+
Hypoxiaprobe
Renal Cortex
Lung
Heart
f
H&E

159

Fig. 3

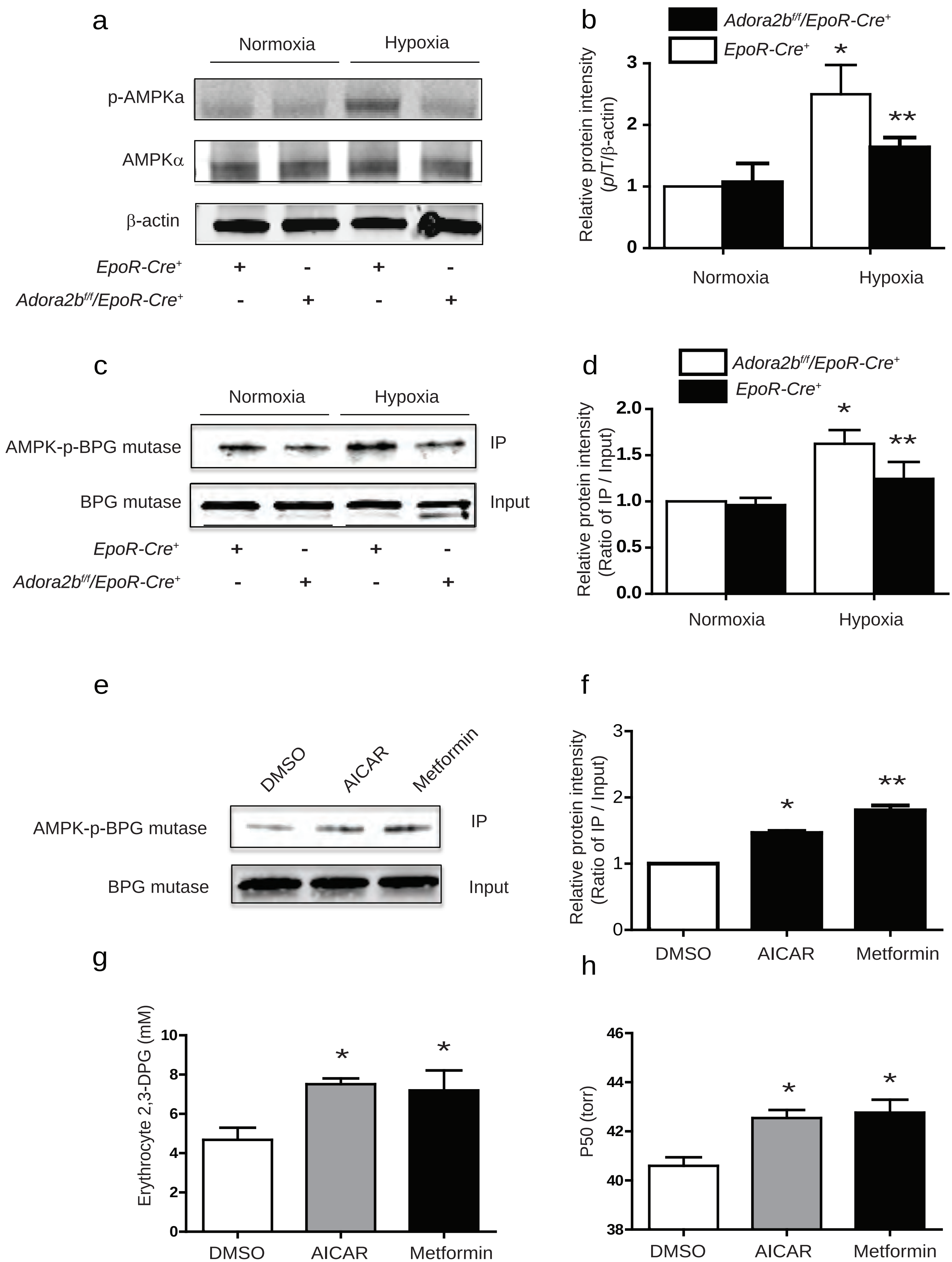

a
Normoxia
Hypoxia
p-AMPKa
AMPKα
β-actin
EpoR-Cre+
Adora2bf/f/EpoR-Cre+
b
Adora2bf/f/EpoR-Cre+
EpoR-Cre+
Relative protein intensity (p/T/β-actin)
Normoxia
Hypoxia
c
Normoxia
Hypoxia
AMPK-p-BPG mutase
BPG mutase
IP
Input
EpoR-Cre+
Adora2bf/f/EpoR-Cre+
d
Adora2bf/f/EpoR-Cre+
EpoR-Cre+
Relative protein intensity (Ratio of IP / Input)
Normoxia
Hypoxia
e
DMSO
AICAR
Metformin
AMPK-p-BPG mutase
BPG mutase
IP
Input
f
Relative protein intensity (Ratio of IP / Input)
DMSO
AICAR
Metformin
g
Erythrocyte 2,3-DPG (mM)
DMSO
AICAR
Metformin
h
P50 (torr)
DMSO
AICAR
Metformin

Fig. 4

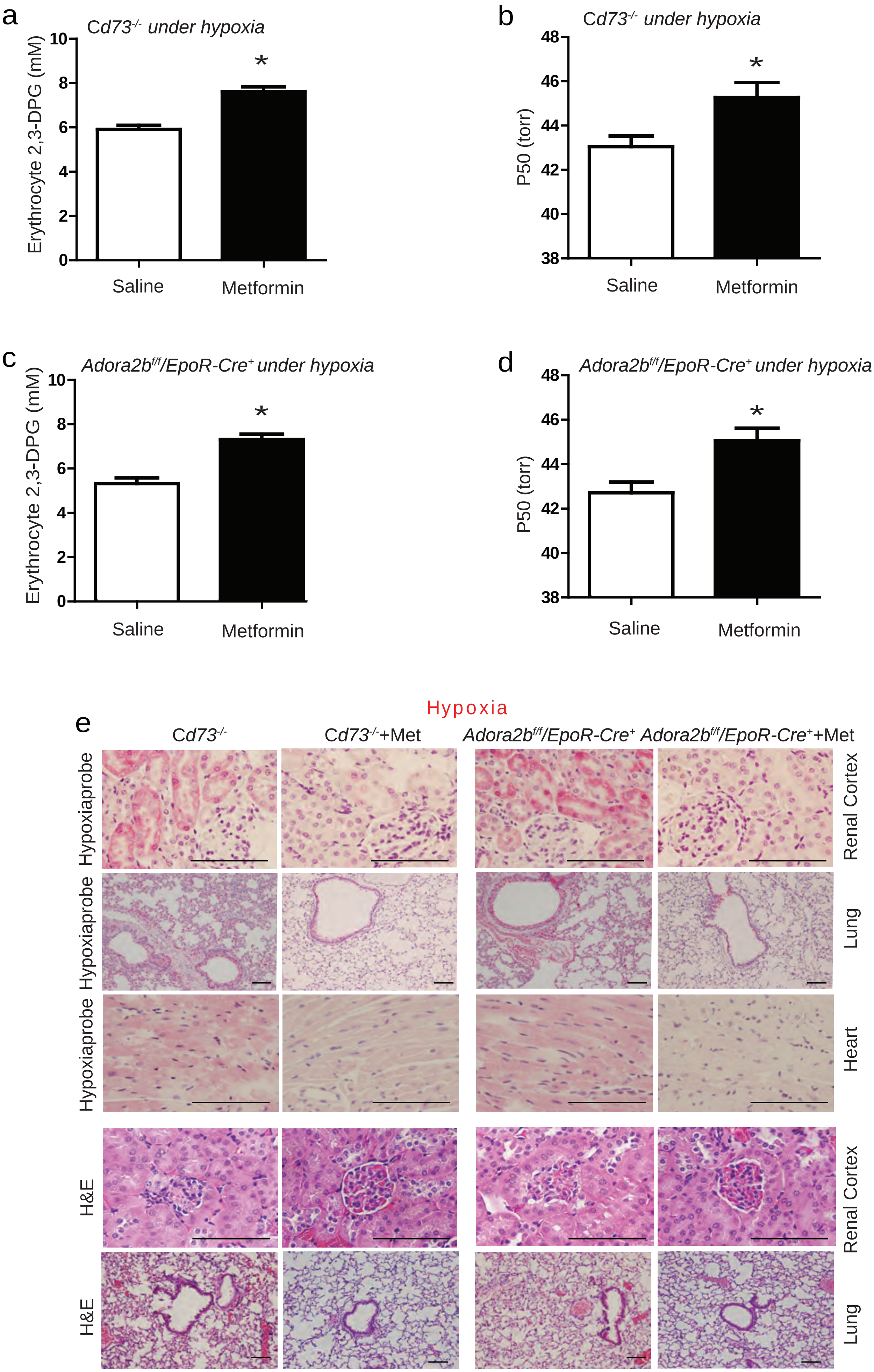

a
Cd73-/- under hypoxia
Erythrocyte 2,3-DPG (mM)
10
8
6
4
2
0
*
Saline
Metformin
b
Cd73-/- under hypoxia
P50 (torr)
48
46
44
42
40
38
*
Saline
Metformin
c
Adora2bf/f/EpoR-Cre+ under hypoxia
Erythrocyte 2,3-DPG (mM)
10
8
6
4
2
0
*
Saline
Metformin
d
Adora2bf/f/EpoR-Cre+ under hypoxia
P50 (torr)
48
46
44
42
40
38
*
Saline
Metformin
Hypoxia
e
Cd73-/-
Cd73-/-+Met
Adora2bf/f/EpoR-Cre+
Adora2bf/f/EpoR-Cre++Met
Hypoxiaprobe
Hypoxiaprobe
Hypoxiaprobe
H&E
H&E
Renal Cortex
Lung
Heart
Renal Cortex
Lung

Fig. 5

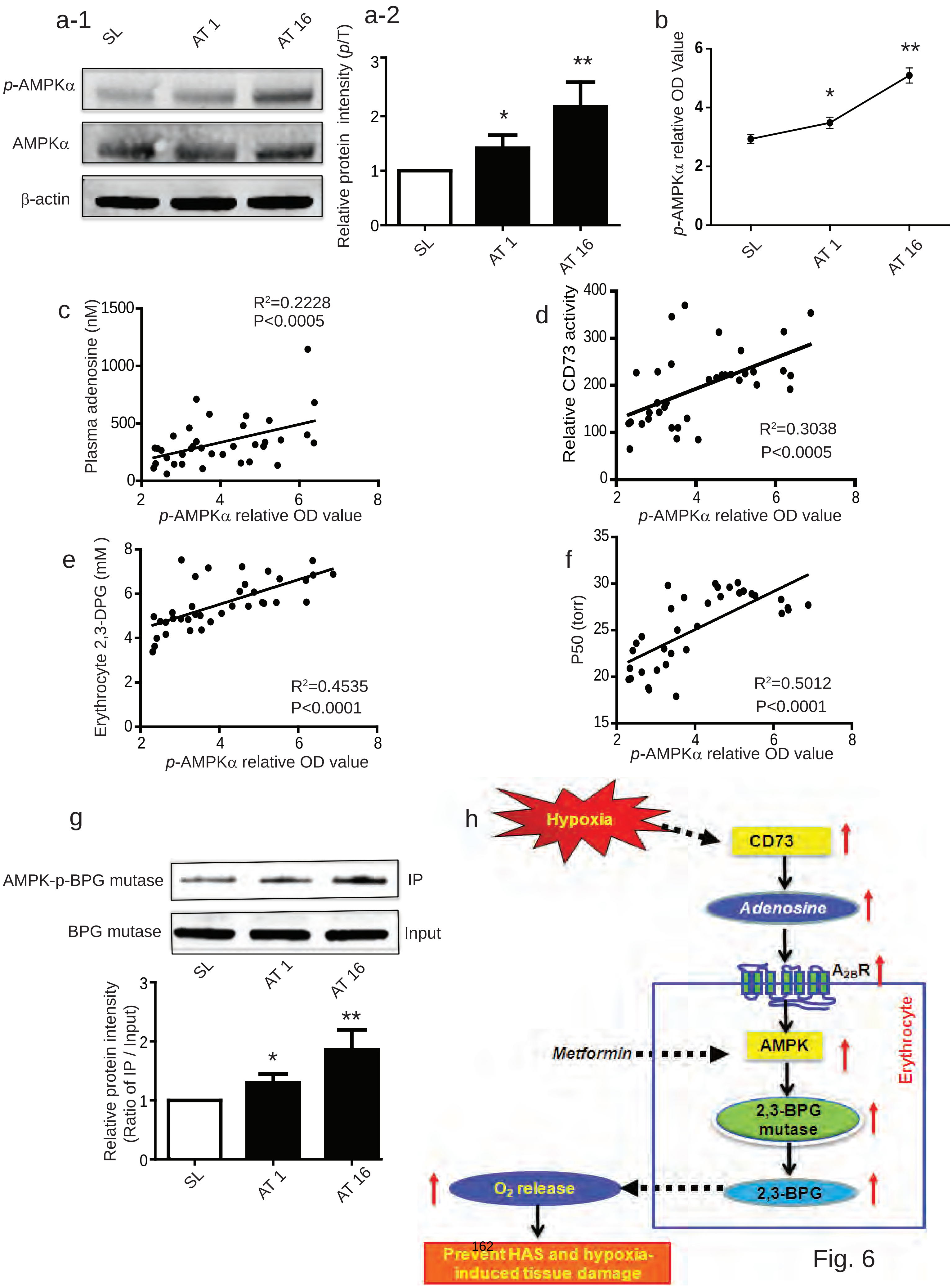
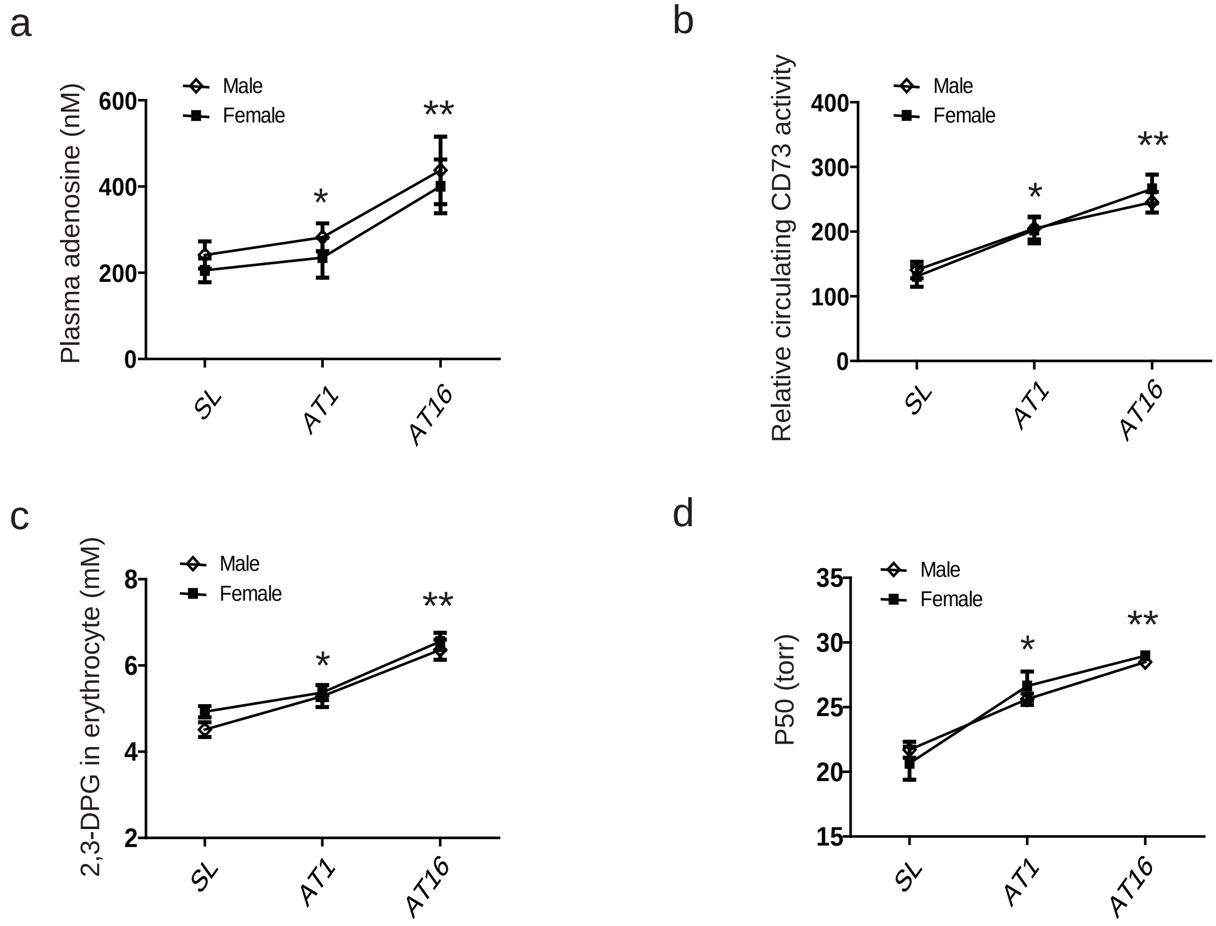
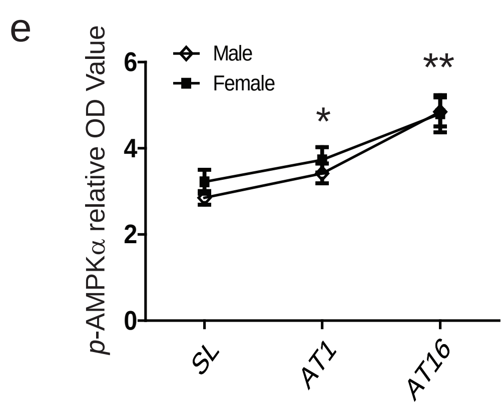

a-1
SL
AT 1
AT 16
p-AMPKα
AMPKα
β-actin
a-2
Relative protein intensity (p/T)
*
**
SL
AT 1
AT 16
b
p-AMPKα relative OD Value
*
**
SL
AT 1
AT 16
c
Plasma adenosine (nM)
R²=0.2228
P<0.0005
p-AMPKα relative OD value
d
Relative CD73 activity
R²=0.3038
P<0.0005
p-AMPKα relative OD value
e
Erythrocyte 2,3-DPG (mM)
R²=0.4535
P<0.0001
p-AMPKα relative OD value
f
P50 (torr)
R²=0.5012
P<0.0001
p-AMPKα relative OD value
g
AMPK-p-BPG mutase
IP
BPG mutase
Input
SL
AT 1
AT 16
Relative protein intensity
(Ratio of IP / Input)
*
**
h
Hypoxia
CD73
Adenosine
A2BR
Erythrocyte
Metformin
AMPK
2,3-BPG mutase
2,3-BPG
O2 release
Prevent HAS and hypoxia-induced tissue damage

Fig. 6



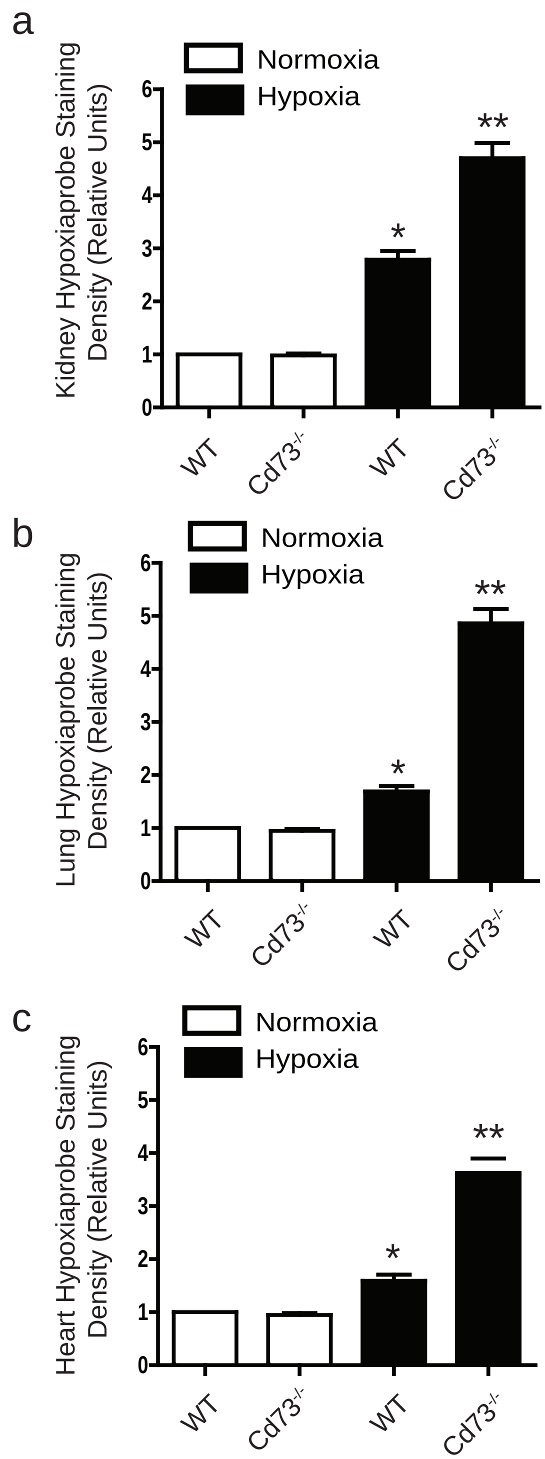


f

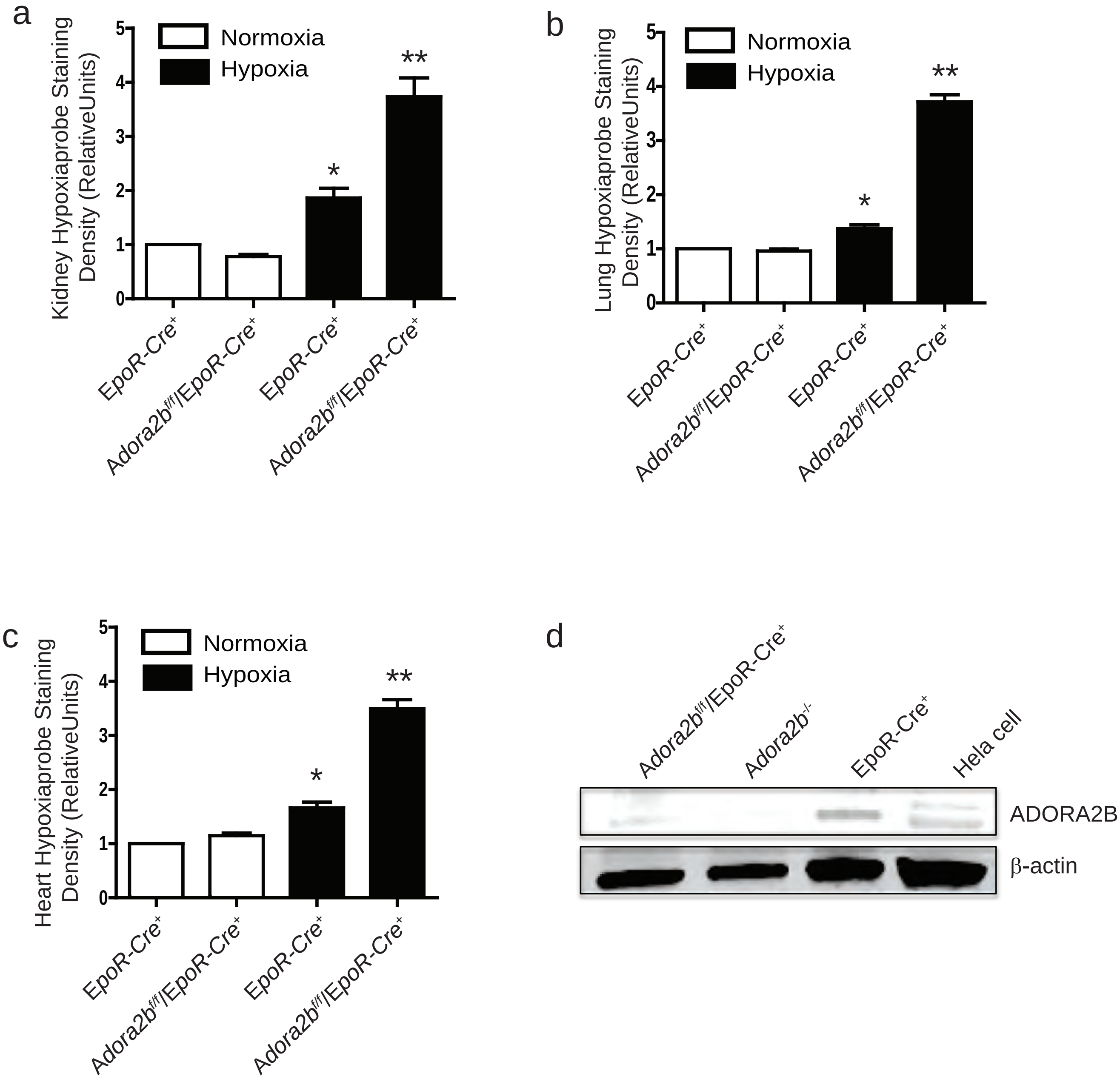
	Number	Age (years)	HT (cm)	WT (kg)	BMI (kg/m^2)
Male	12	21.08±1.24	181.30±4.06	75.29±6.26	22.85±1.67
Female	9	20.44±1.51	168.48±5.18	62.31±6.43	21.88±1.84

Supplementalry Figure 1. No significant differences were observed between males and females of humans at high altitude in elevated plasma adenosine levels, elevated soluble CD73 activity, elevated erythrocyte 2,3-BPG level, elevated O_2 release capacity of RBC, and elevated p-AMPK level of erythrocyte

a-e Plasma adenosine levels, soluble CD73 activity, erythrocyte 2,3-BPG level, O_2 release capacity of RBC, and erythrocyte p-AMPK level were significantly elevated both in male and female at high altitude, and there was no significant different between male and female.Data are expressed as mean ± SEM; *P<0.05 vs sea level; **P<0.05 vs ALT1. **f.** General Subject Characteristics.

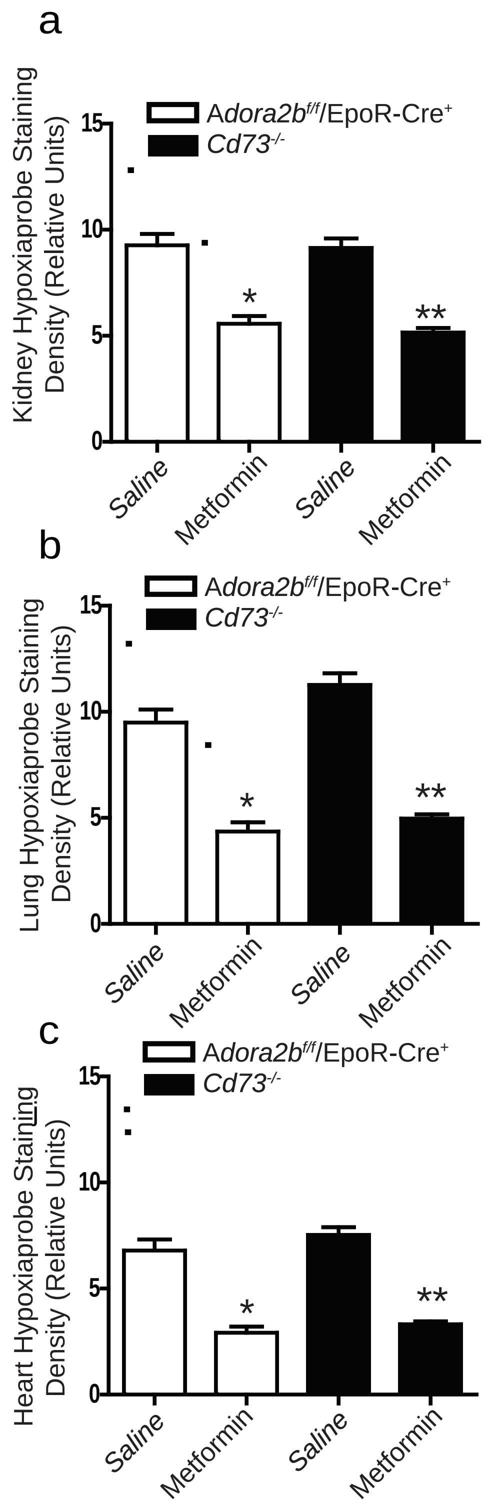


Supplementary Figure 2. Deletion of CD73 result in severe multiple tissue hypoxia
a-c Quantification of the immunohistochemical staining was performed using the Image-Pro Plus software, showing tissue hypoxia level were significantly induced in lung, kidney and heart of WT mice post hypoxic treatment, and it were further induced in the tissue of kidney, lung and heart in C*d73*$^{-/-}$ mice post hypoxic treatment. Data are expressed as mean ± SEM; *P<0.05 vs WT under normoxia condition; **P<0.05 vs WT under hypoxic condition. (n=8-10 mice per group)

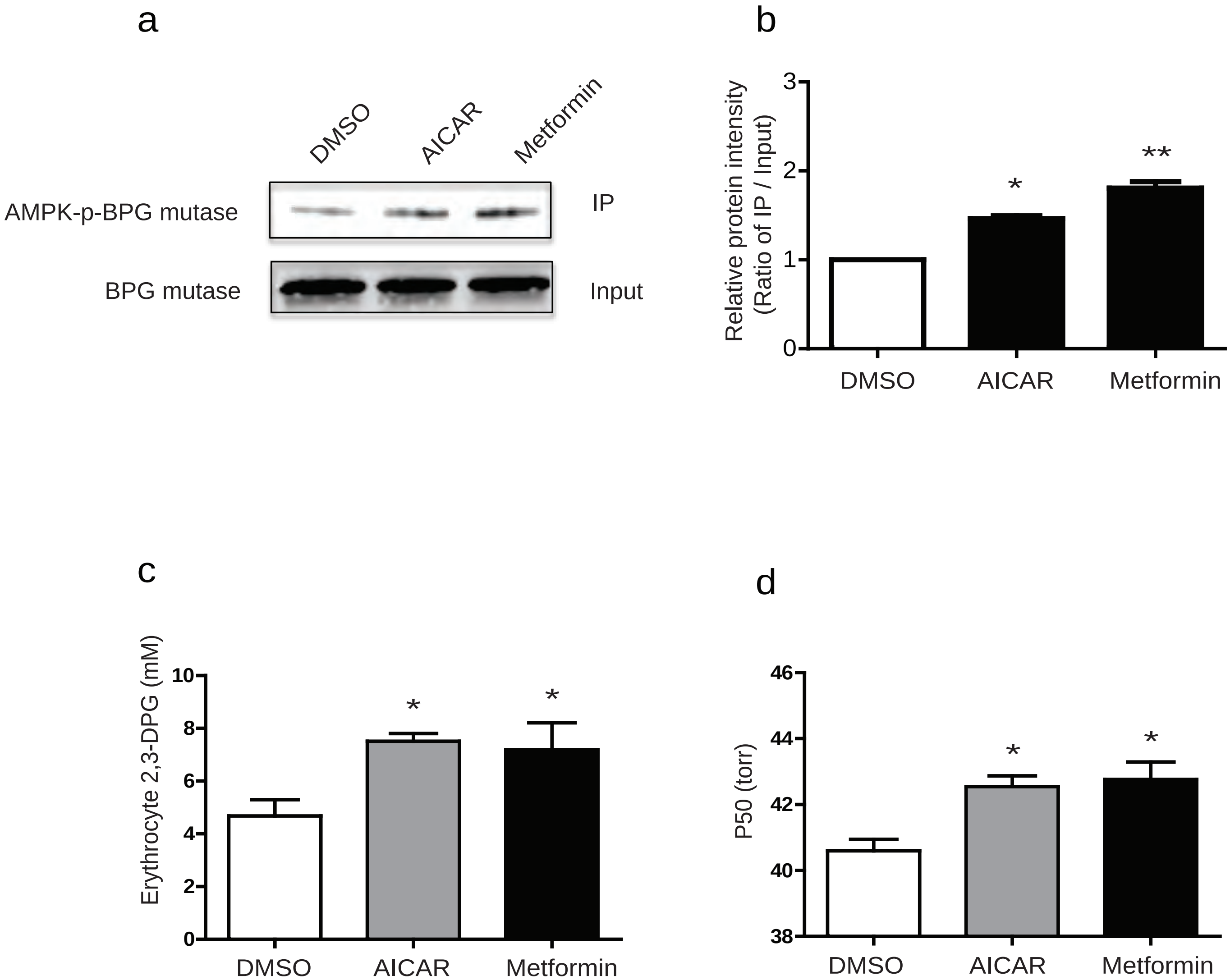


Supplementary Figure 3. Targeted deletion of ADORA2B in erythrocyte result in severe multiple tissue hypoxia

a-c Quantification of the immunohistochemical staining showed that tissue hypoxia level were significantly induced in lung, kidney and heart of E*poR-Cre*$^{+}$ mice post hypoxic treatment, and it were further induced in the tissue of kidney, lung and heart in A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice post hypoxic treatment.
*P<0.05 vs E*poR-Cre*$^{+}$ mice under normoxia condition; **P<0.05 vs E*poR-Cre*$^{+}$ mice under hypoxic condition (n=8-10 mice per group). **d.** ADORA2B protein levels of erythrocytes in A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice, A*dora2b*$^{-/-}$ mice and E*poR-Cre*$^{+}$. Hele cell as postive control.



Supplementary Figure 4. ***In vivo* effects of metformin treatment on multiple tissue hypoxia**
a-c Quantification of the immunohistochemical staining showed that metformin treatment significantly attenuated tissue hypoxia level in lung, kidney and heart of A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice and C*d73*$^{-/-}$ mice post hypoxia. Data are expressed as mean ± SEM; *P<0.05 vs A*dora2b*$^{f/f}$/E*poR-Cre*$^{+}$ mice with saline treatment; **P<0.05 vs WT C*d73*$^{-/-}$ mice with saline treatment. (n=6-8 mice per group)



Supplementary Figure 5. **AMPK agonists directly induce activation of erythrocyte AMPK and its phosphorylation of 2,3-BPG mutase, result in 2,3-BPG production and P50 in cultured primary mouse erythrocytes *in vitro*.**

(a)Representative western blot analysis of phosphorylated 2,3-BPG mutase levels in lysates of DMSO, Metformin or AICAR-treated primary mouse erythrocytes immunoprecipated by an antibody specific for phosphorylated AMPK substrates. (b)Image quantification analysis showing that levels of *p*-AMPK-mediated phosphorylation of 2,3-BPG mutase were significantly induced by metformin or AICAR treatment in the cultured primary mouse erythrocytes (n=6-8). AMPK agonists directly induce both Metformin and AICAR directly induced 2,3-BPG concentrations (c) and P50 (d) in cultured primary erythrocytes isolated from wild type mice. Data are expressed as mean ± SEM; *P<0.05 for Metformin or AICAR treated mouse erythrocytes vs DMSO-treated cells (n=6-8 mice per group).

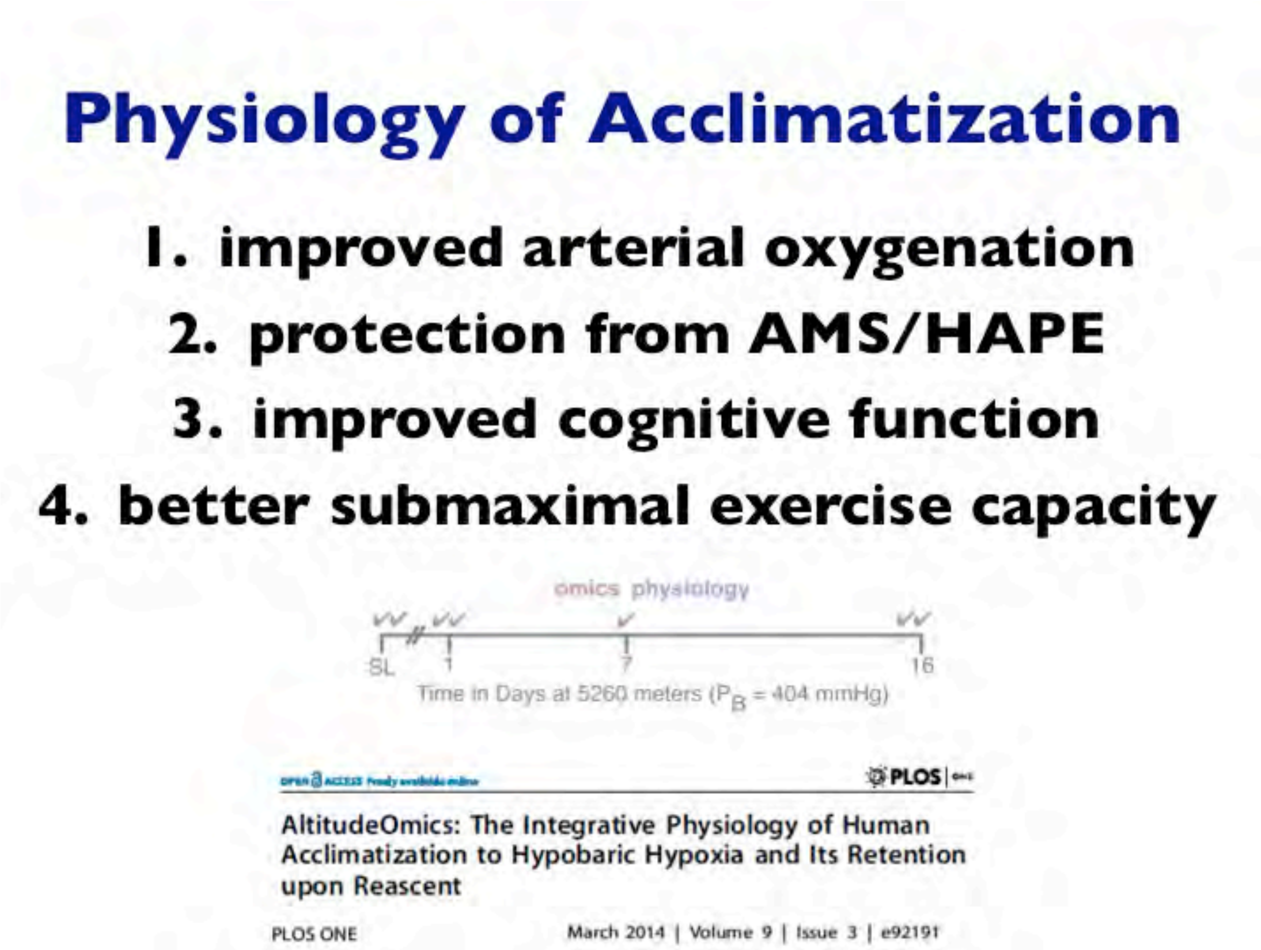
transcriptomic and epigenomic mechanisms of human adaptation to hypoxia

University of Colorado
Altitude Research Center

by Robert Roach and the AltitudeOmics Team

This draft document summarizes the outline for the work-in-progress manuscript on the gene expression and epigenetic changes we observed during the process of acclimatization. We expect to complete this paper by the end of 2014.

RR Friday, October 31, 14



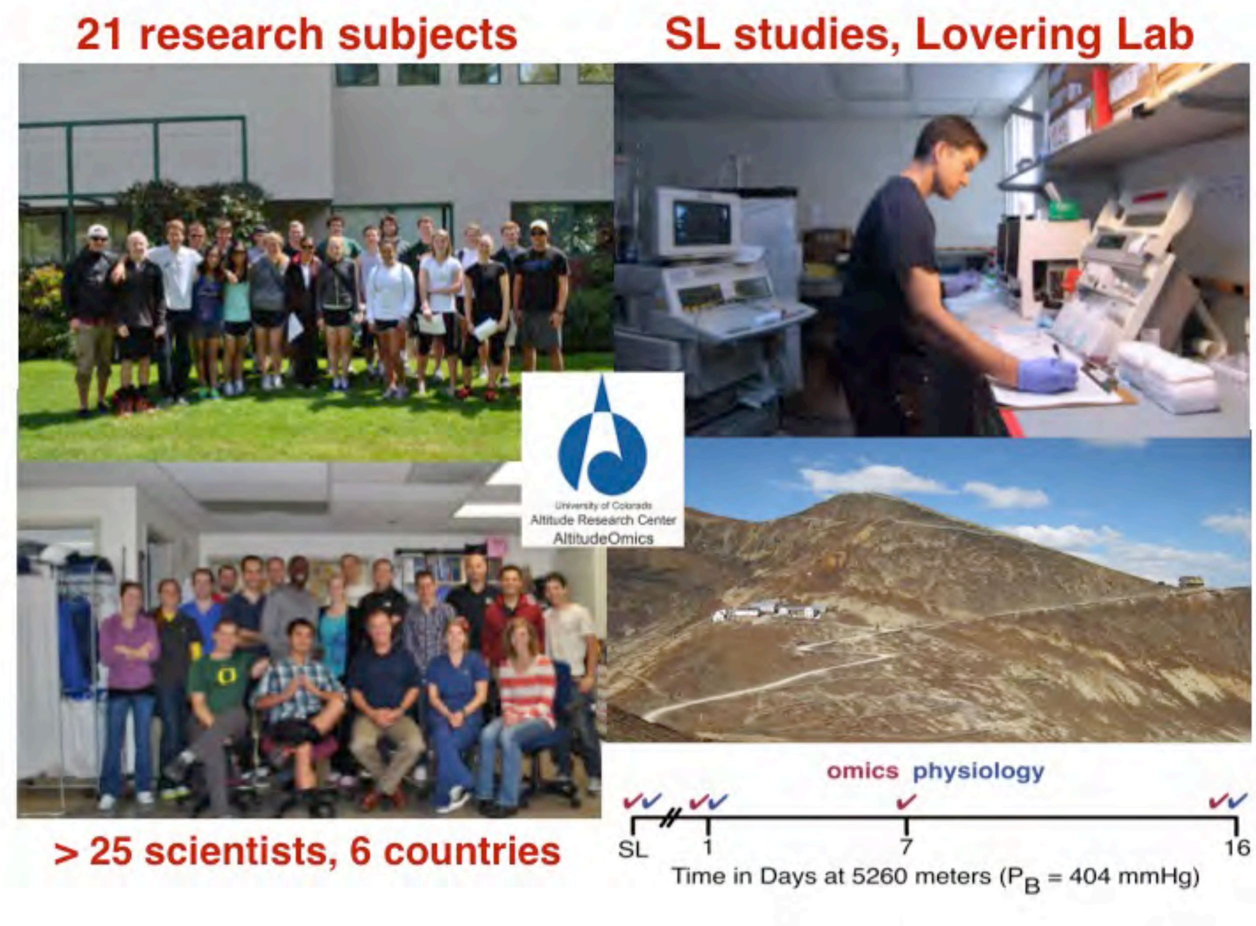
The purpose of this study was to discover the basic molecular and cellular mechanisms controlling human adaptation to hypoxia to lead us to new approaches to improving the process of human adjustment to hypoxia for soldiers, patients and high altitude residents.

AltitudeOmics

The Integrative Physiology of the Onset and Retention of Acclimatization to Hypoxia in Humans

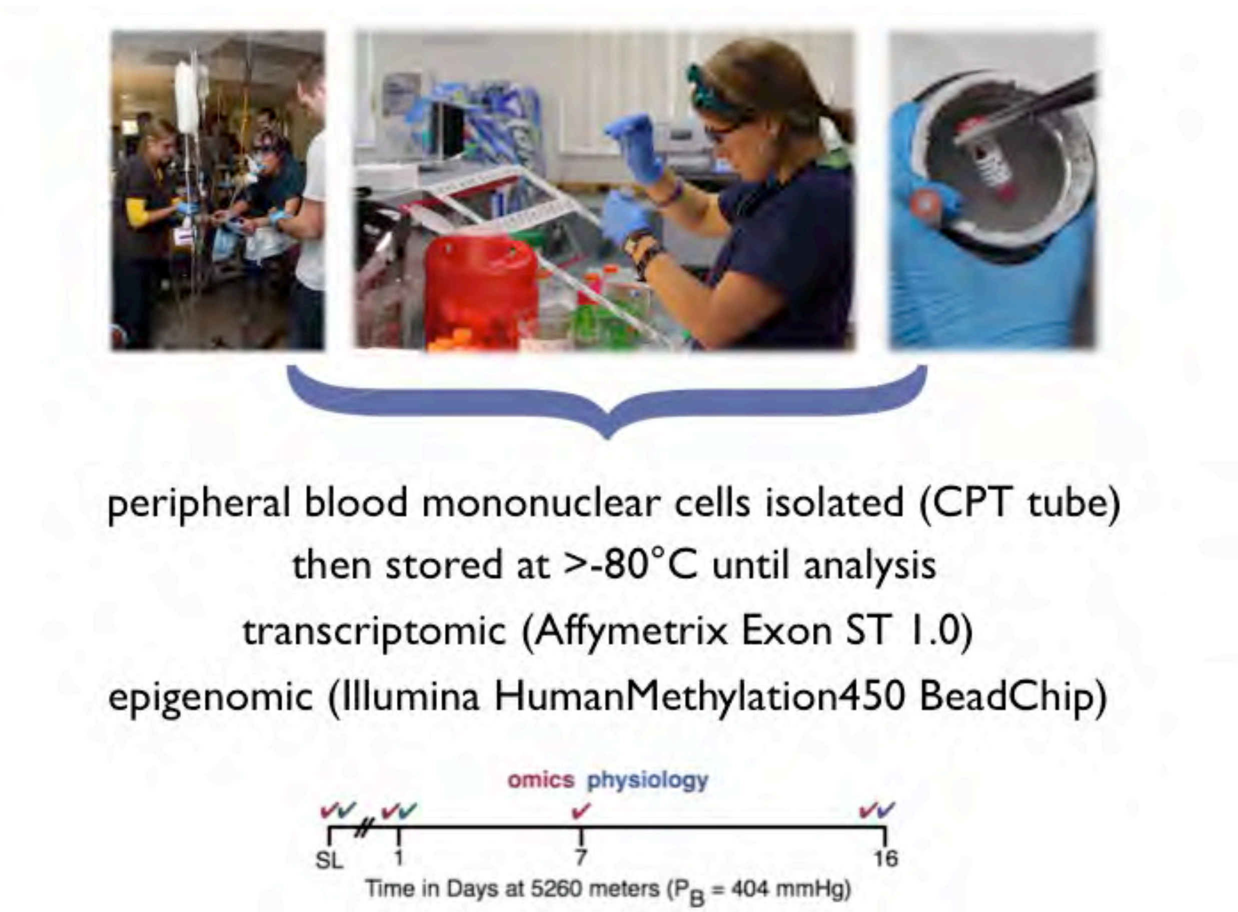
over 25 scientists from 6 countries

In the AltitudeOmics study we leveraged the support of the DoD for the basic study, brought in more than two-dozen internationally acclaimed investigators, added more than a dozen additional studies, and accomplished all of our objectives in a timely manner.

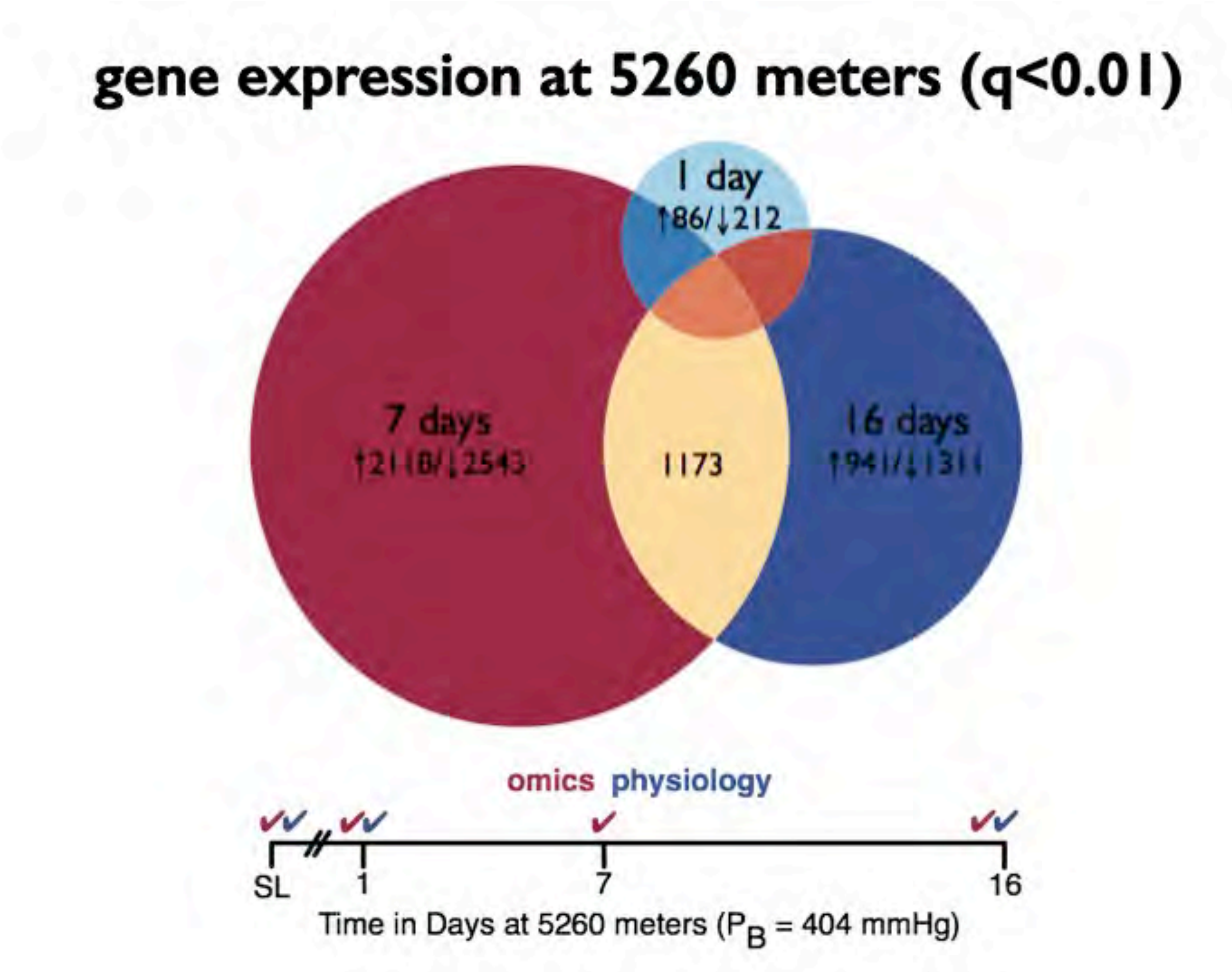


Dr. Andrew Lovering, another TATRC funded investigator who happens to be a close friend and colleague agreed to help recruit subjects for us form sea level. We originally did not have enough funding to get subjects from elsewhere, but through budget tightening, the donation by Dr. Lovering of his team and laboratory for months of baseline work and help in the field made this strong study even better by letting us have sea level subjects (instead of subjects from our lab are oat moderate altitude in Denver).

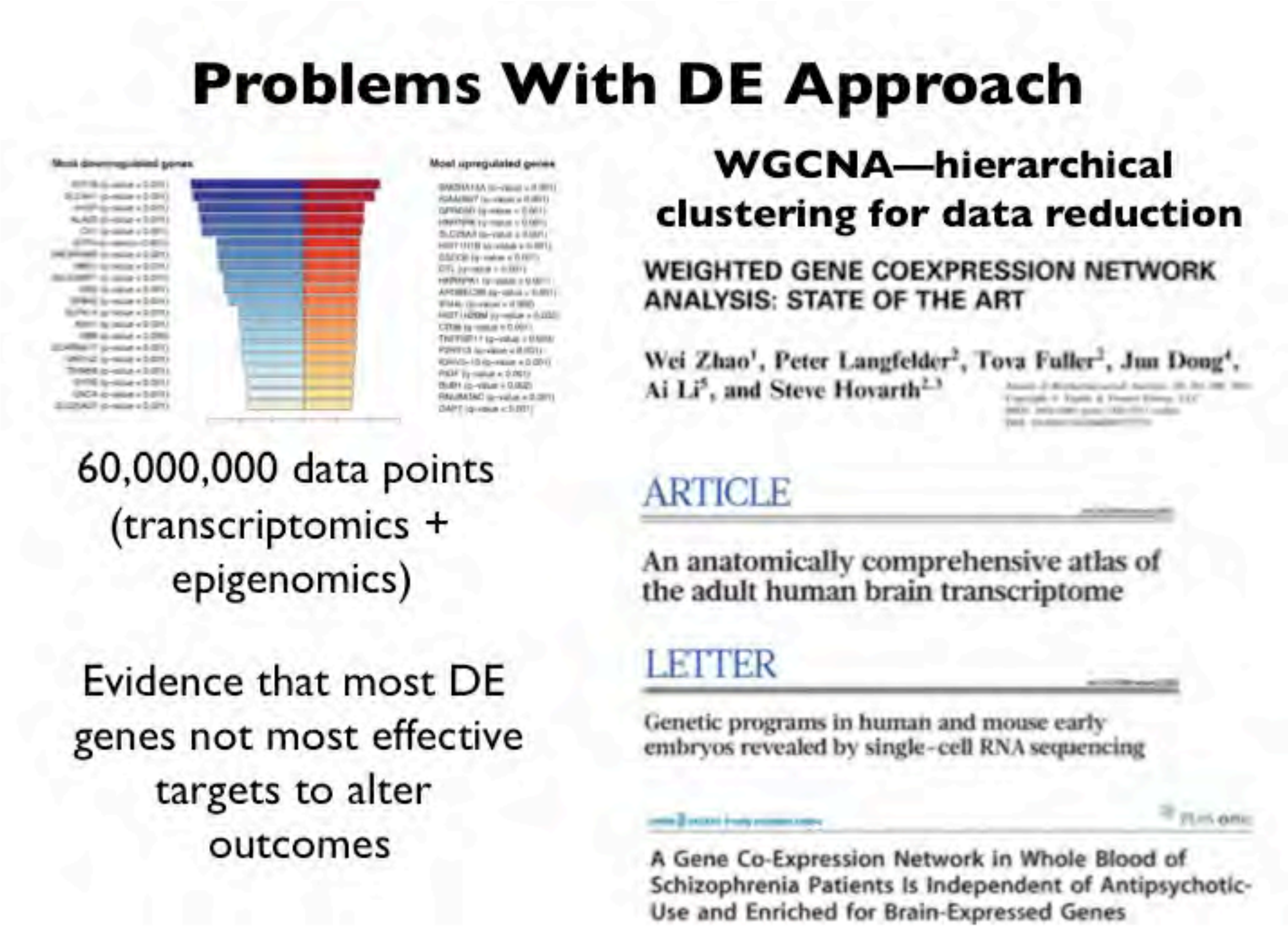
The 24 subjects and 26 scientists and additional numerous volunteers and helpers in Denver, Eugene and Bolivia contributed to make this challenging project a great success.



Samples for the transciptomic and epigenomic part of the study were collected using identical protocols at low and high altitude. Data was analyzed on return to Denver using standard chip-based assays.

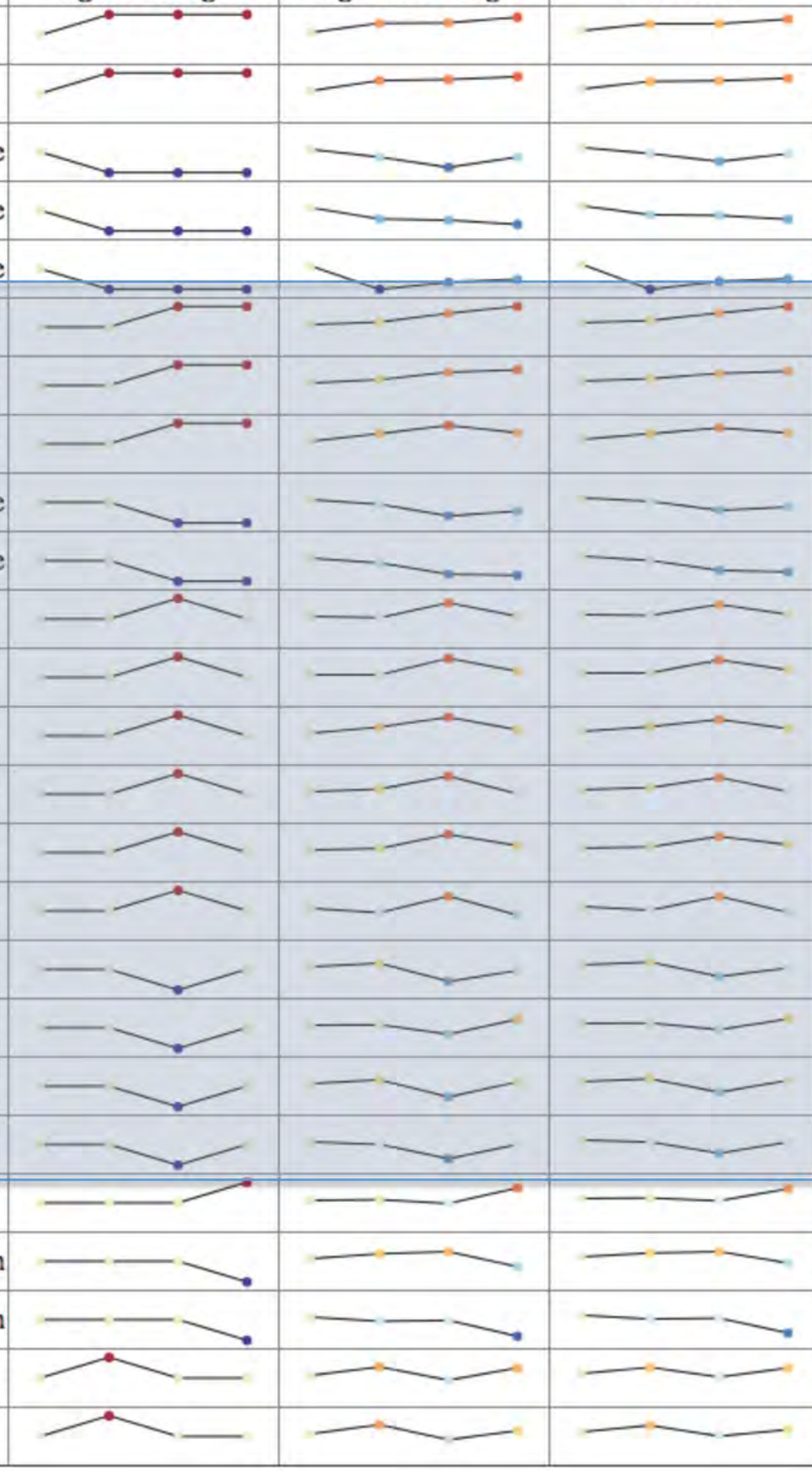


The simplest way to look at gene expression is to examine the number of genes that are up regulated and down regulated, and in our case, the time points when those changes occur. Thousands of genes changed at the 7th day, and also on the 16th day of the present study.

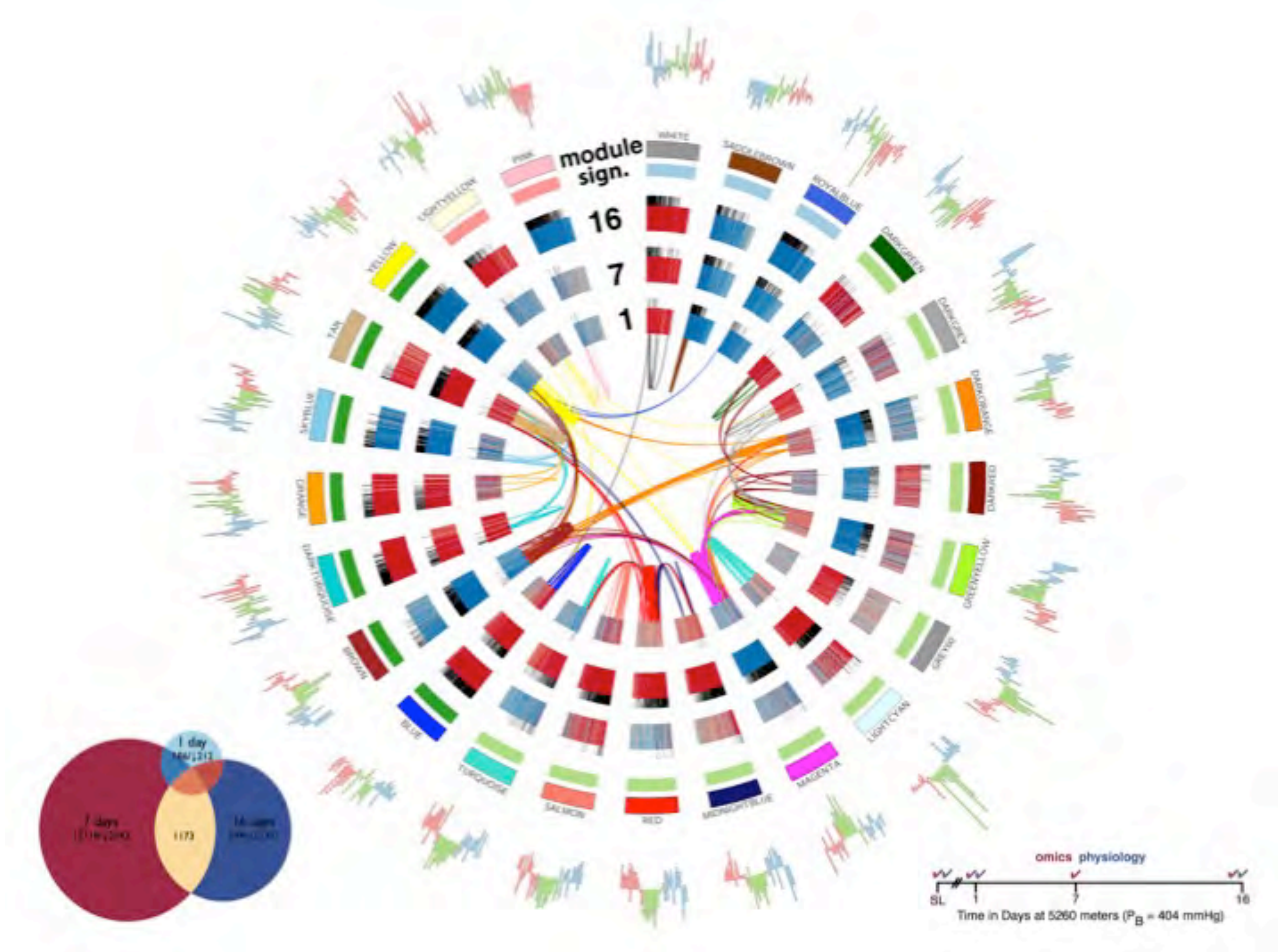


But knowing that a lot of genes have changed can lead you on a wild goose chase to determine which of those thousands of changes are meaningful. Recently scientists have begun using clustering techniques to group genes that change together into modules of genes. This greatly reduces the number of genes you have to be concerned with comparing, thus making your planned comparisons much more powerful.

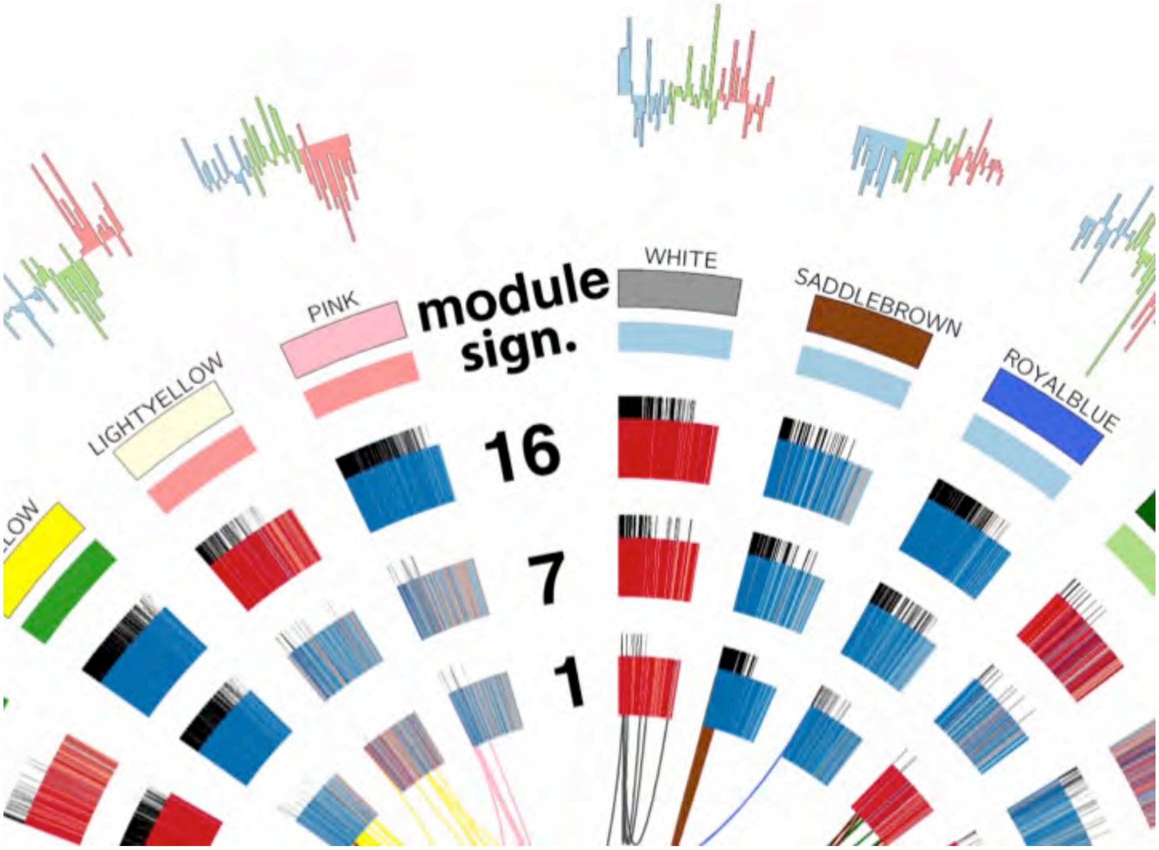
name	pattern	signed change	log2 fold change	t-statistic
darkturquoise	up same same			
white	up same same			
brown	down same same			
royalblue	down same same			
saddlebrown	down same same			
blue	same up same			
orange	same up same			
tan	same up same			
skyblue	same down same			
yellow	same down same			
grey60	same up down			
lightcyan	same up down			
midnightblue	same up down			
red	same up down			
salmon	same up down			
turquoise	same up down			
darkorange	same down up			
darkred	same down up			
greenyellow	same down up			
magenta	same down up			
lightyellow	same same up			
grey	same same down			
pink	same same down			
darkgreen	up down same			
darkgrey	up down same			



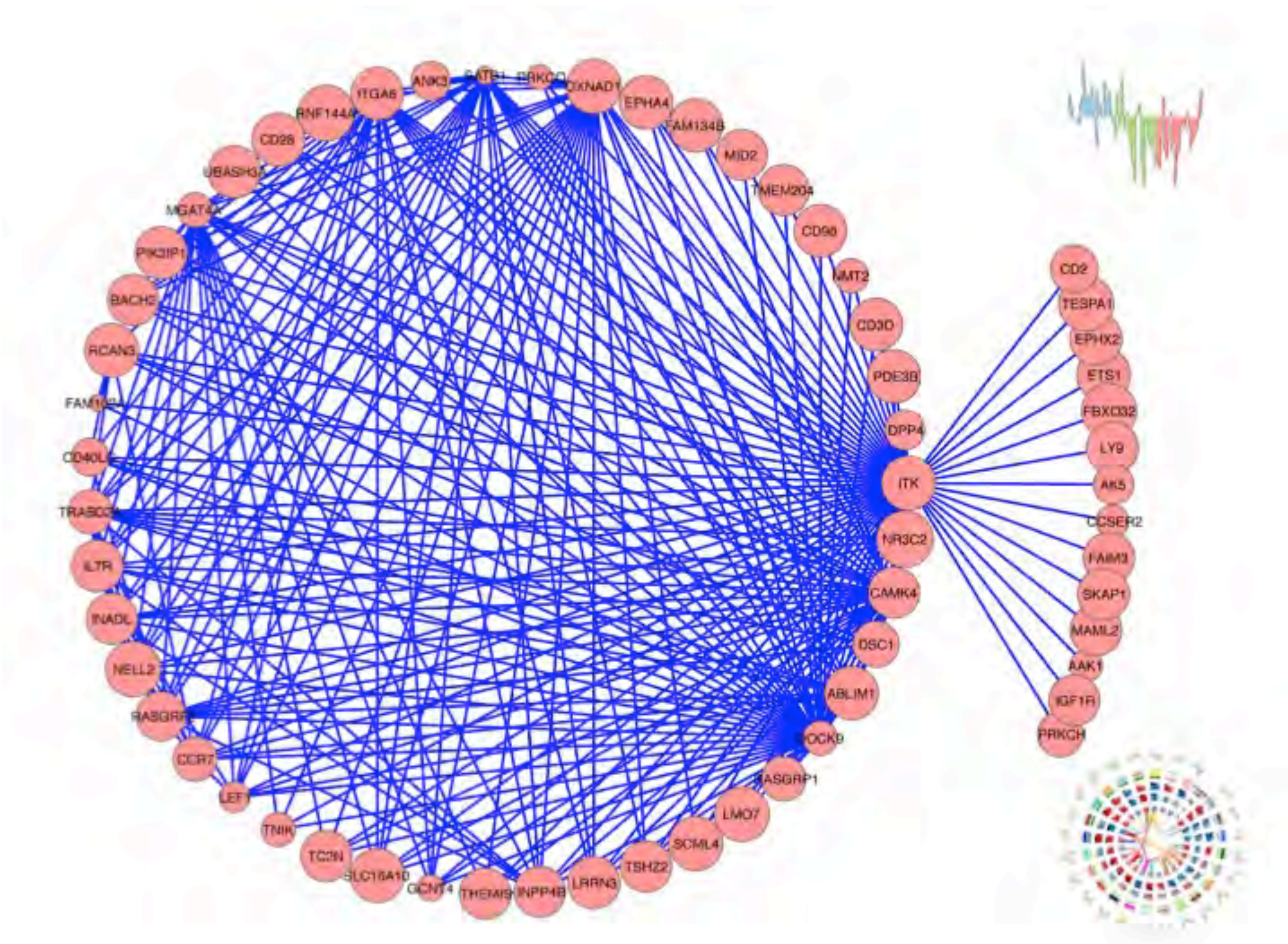
We used this clustering approach and discovered 15 modules or groups of genes that have major changes (up or down) on the 7th day at high altitude. We think that these genes are the key genes for initiating acclimatization.



All the genes in all the modules at all the time points can be represented in one graphic as shown here. The next page has a blow up of the graph that explains the details illustrated.

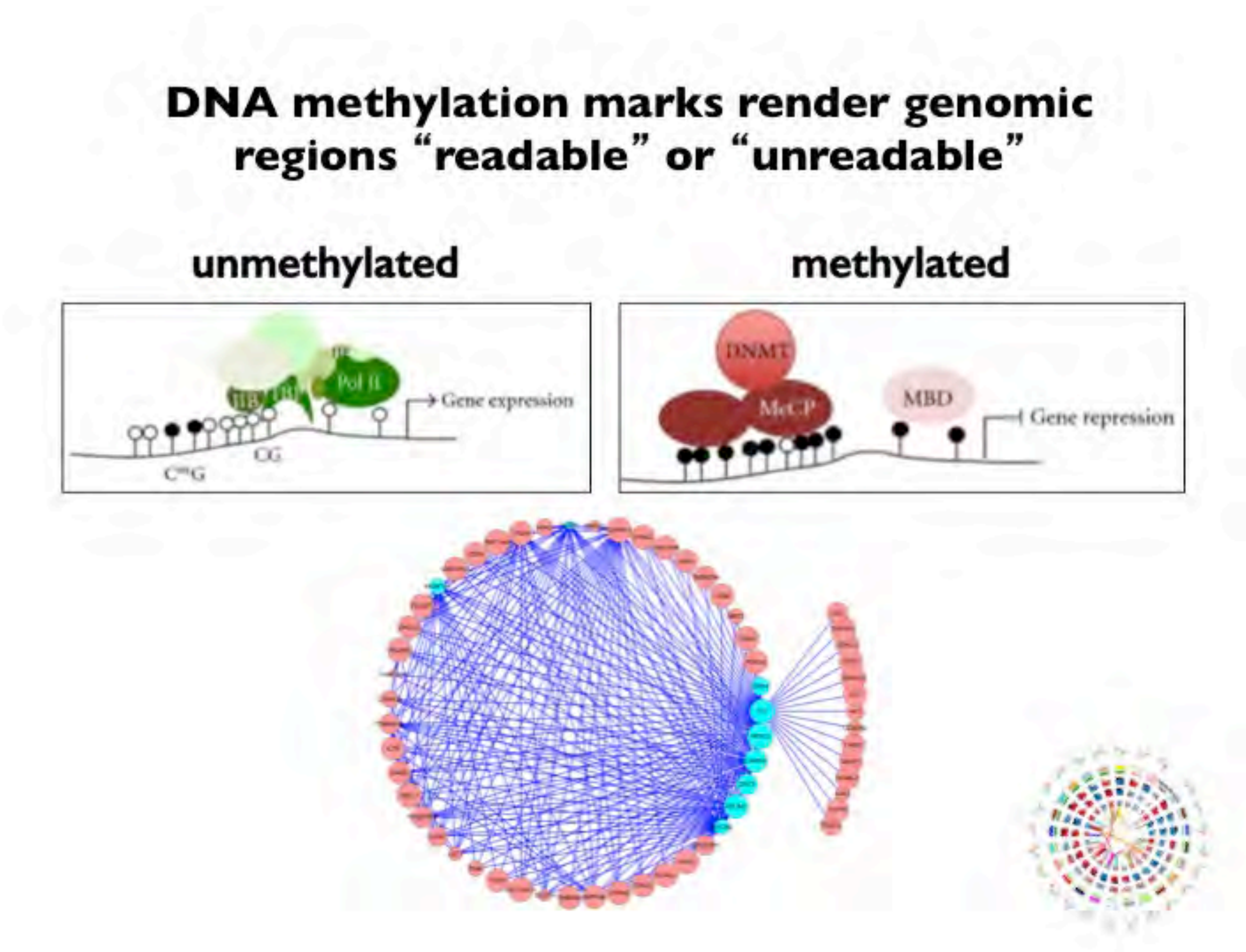


The center of the circus diagram shows inter-module transcription factor connections. In the #1 ring is show the gene expression for all the genes in each module on altitude day 1 compared to sea level, and so on for 7 and 16. Then the colored bar represents the time period when each module is significantly different than sea level. And the most outer band is the module eigengene showing a representative value for each person for all their genes that are in each module.

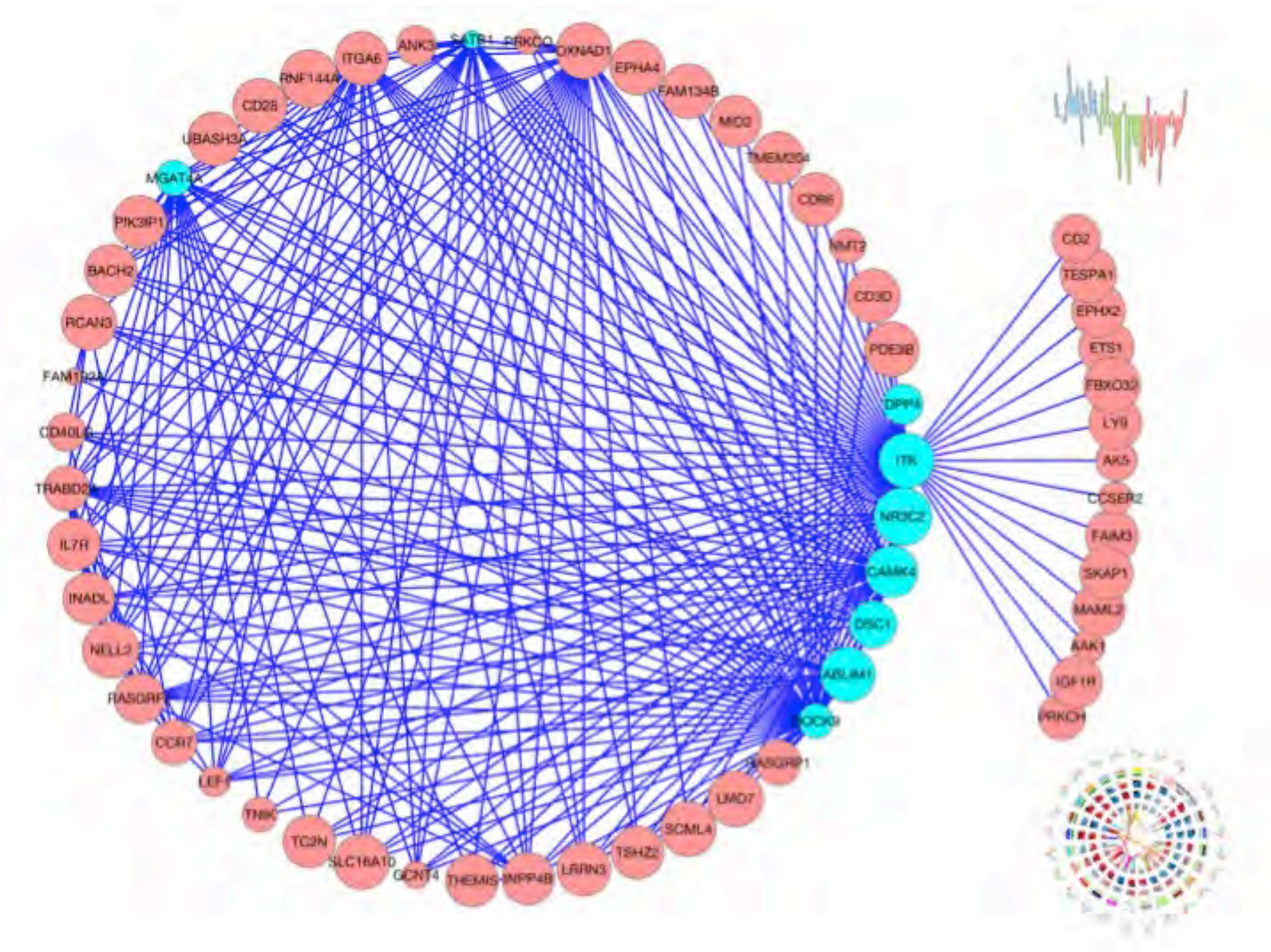


As an example of the power of this approach we picked the yellow module that contains the genes featured above as hub genes, or the most central genes in the module. This module is one of the modules that changes during acclimatization, and is proposed to contain genes that control parts of the acclimatization process.

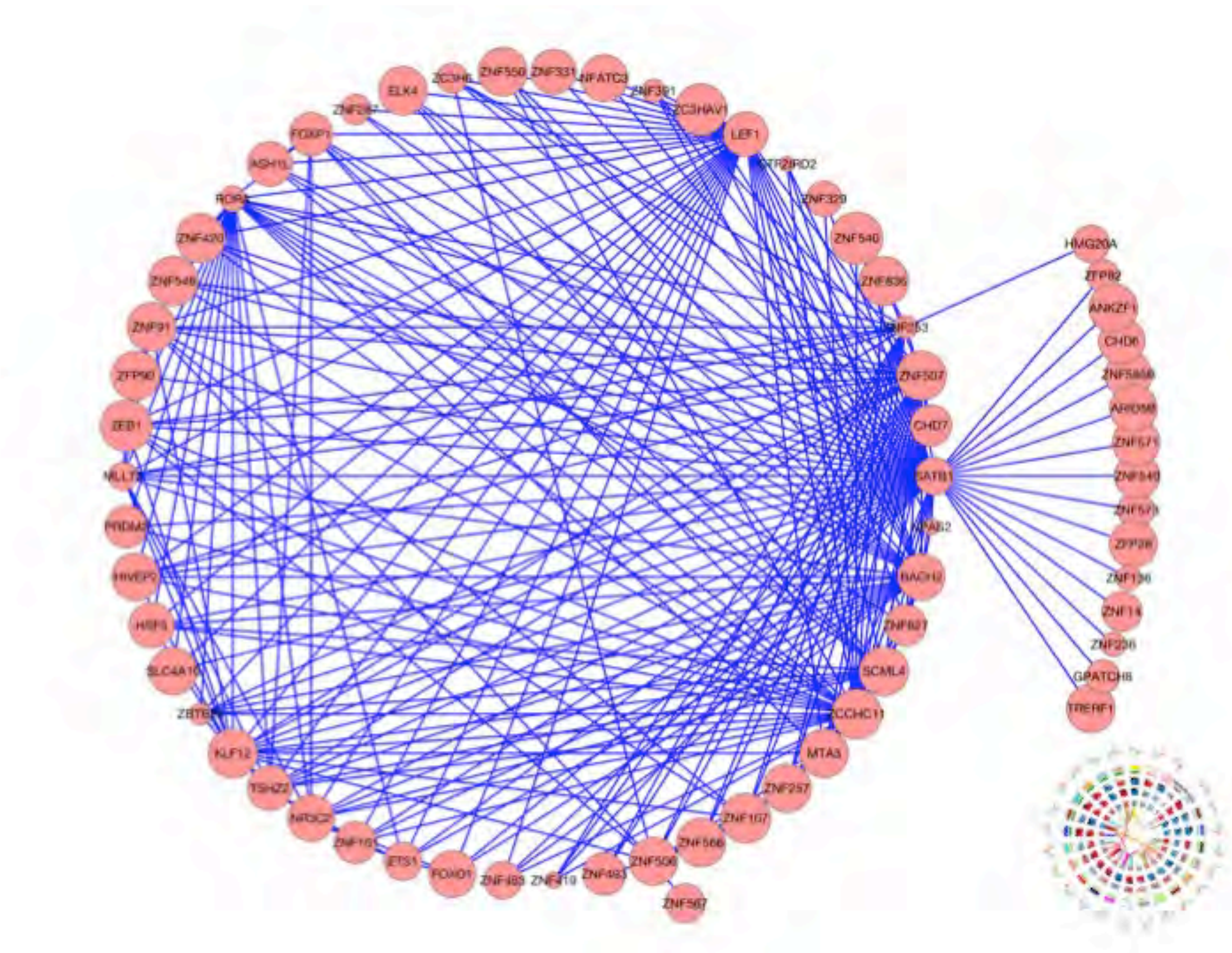
One of the exciting aspects of the module-centric approach is that well-established network theory suggests that disrupting the hubs, much as you might disrupt a transportation hub, will cause the greatest perturbation in the network. In this case it would suggest that ITK as one of the most central hubs in the yellow module might be central to the process of acclimatization.



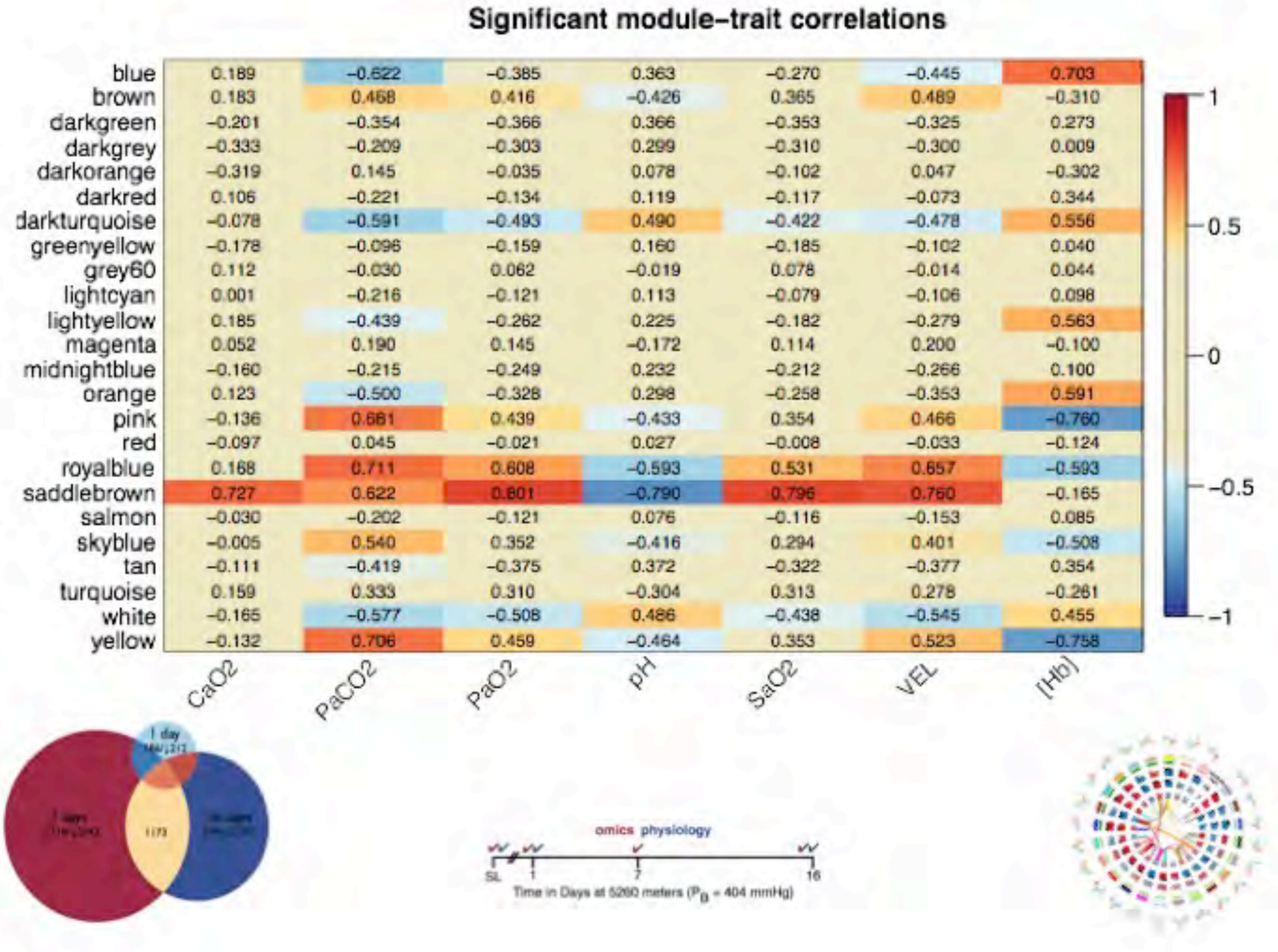
An additional component of the omic signaling pathway is to consider whether one of the key genes is epigentically modified thus potentially leading to an easily modifiable gene process that could improve acclimatization. In the yellow module we identified a handful of genes that were epigentically modified as shown in the next page.



The genes shown above in turquoise are epigenetically hypermethylated during acclimatization. These findings suggest that these genes are key to acclimatization. And the nature of hypermethylation suggests that animal models can be rapidly developed and tested for manipulation of the genes as they relate to acclimatization.



We can also identify in a given module the transcription factors that may be controlling part of the response of each module. Shown above are the transcription factors that are significant players in the yellow module. Each of these transcription factors would be a reasonable target for further investigation in animal studies.



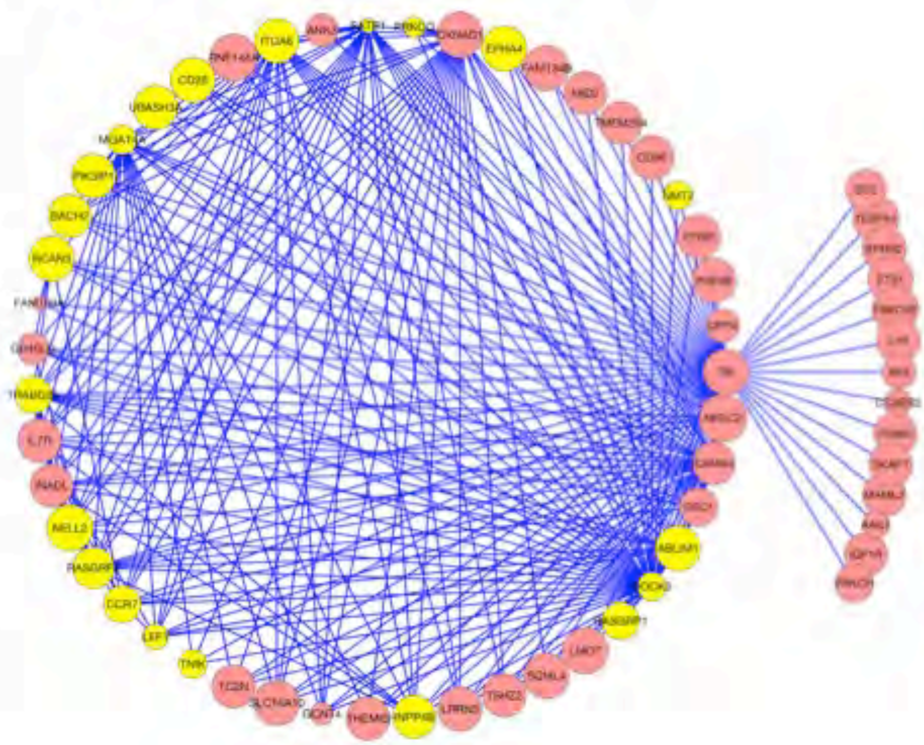
Condensing the ~23,000 genes measured for each of the 21 subjects at sea level, twice on the first day, on the 7th and 16th days at altitude into modules and then using the representative value for the behavior of that module, the eigengene we can correlate that single value to any trait we have measured in the subjects for insights into gene-to-trait relationships. Shown above is a simple correlation matrix of all module eigengenes against a number of key traits in the AltitudeOmics study. Darker colors highlight significant correlations.

Peripheral Blood Mononuclear Cell Gene Expression in Chronic Obstructive Pulmonary Disease

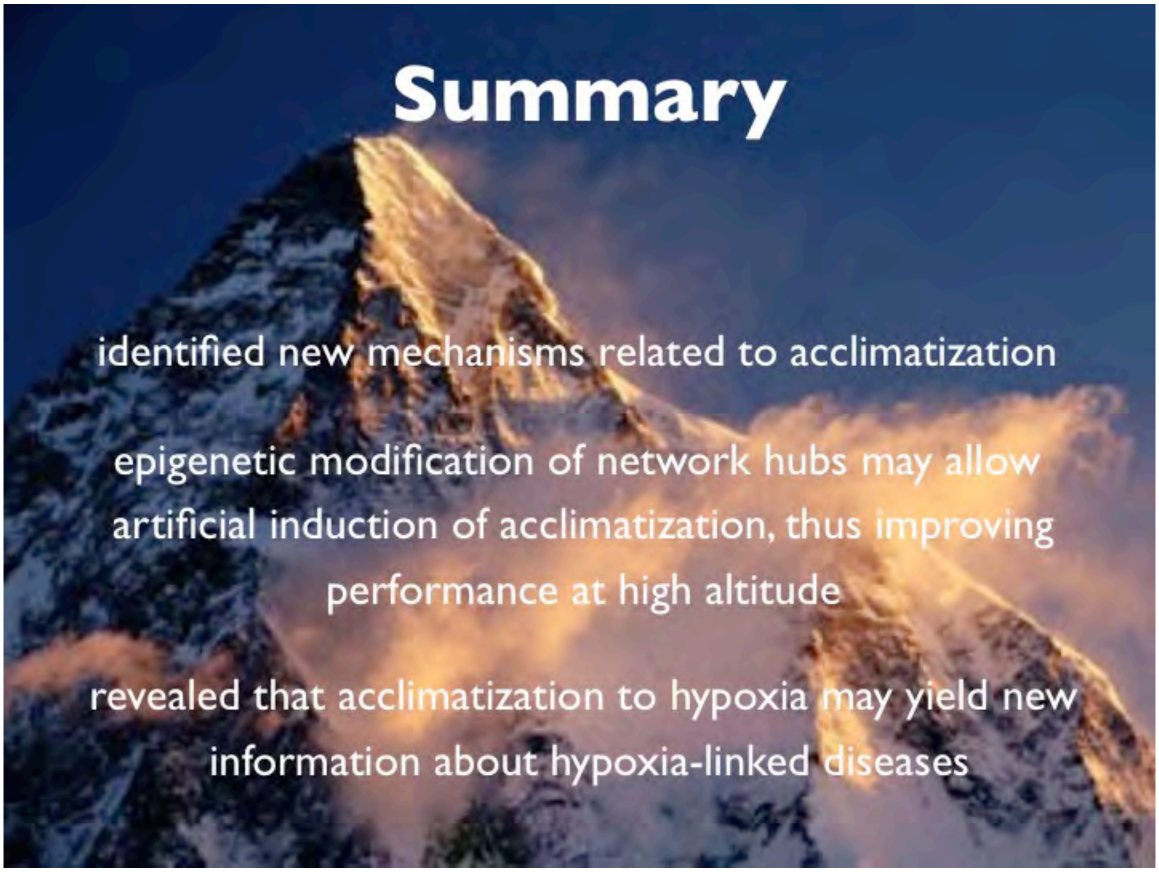
Timothy M. Bahr[1*], Grant J. Hughes[1], Michael Armstrong[4], Rick Reisdorph[3], Christopher D. Coldren[4], Michael G. Edwards[2], Christina Schnell[4], Ross Kedl[3], Daniel J. LaFlamme[5], Nichole Reisdorph[3], Katerina J. Kechris[1†], and Russell P. Bowler[3,4†]

AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY VOL 49 2013

Yellow highlights are genes in common with COPD patients, but expressed in opposite direction as our subjects adjusted to hypoxia



And finally we can look at our modules thought to be responsive to hypoxia and see how they relate to other conditions where humans experience hypoxia. Here highlighted in yellow are genes in the yellow module that are expressed in patients with chronic obstructive pulmonary disease and in our dataset were expressed in the opposite direction. This observation suggests that humans can respond to hypoxia in a positive fashion, but in COPD that response is deficient.



In summary, the AltitudeOmics study made three major breakthroughs. We have identified many hundreds of new mechanisms related to acclimatization, including epigenetic modifications of key hub genes that may be targets suitable for pharmacologic manipulation to improve performance at high altitude and revealed that acclimatization to hypoxia may yield new information about hypoxia-linked diseases.

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Final Announcement

INSIDE

Hosted by the United States Army Research Institute of Environmental Medicine

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Tuesday › 19 August *(continued)*

0900-0915 Break

0915-1115 — **CONCURRENT SYMPOSIA**

Physical Performance, Musculoskeletal Injuries and Women in the Military: State of the Science and Recommendations for the Way Ahead Chair: Bradley Nindl (USA)

Physiological and Medical Aspects That Put Women Soldiers at Increased Risk for Overuse Injuries
Yoram Epstein (Israel)

Risk Factors for Musculoskeletal Injuries in Deployed Female Soldiers
Tanja Roy (USA)

Sex-Specific Applicant Physical Selection Standards May Be Appropriate
Jace Drain (Australia)

Performance Differences on Combat Proxy Tasks in U.S. Marines: Are Females Ready for the Fight?
Karen Kelly (USA)

Physical Training Strategies for Performance Optimization in Women in Combat-Centric Occupations
Bradley Nindl (USA)

Minimal Footwear – A Return to Basics Chair: Irene Davis (USA)

Biomechanical Differences Between Running in Minimal Footwear and Traditional Footwear
Irene Davis (USA)

Injuries with Minimal Footwear Running
Sarah Trager Ridge (USA)

Safe Transitioning to Minimal Footwear Running
Neil Flemming (USA)

AltitudeOmics to Advance Warfighter Performance at High Altitude
Chair: Robert Roach (USA)

The Integrative Physiology of Human Adjustment to High Altitude: Implications for the Warfighter
Robert Roach (USA)

The Integrative Physiology of Breathing at High Altitude: Implications for the Warfighter
Bengt Kayser (Switzerland)

Fatigue at High Altitude: Where Does It Come From and What Can the Warfighter Do About It?
Stewart Goodall (UK)

New Insights into Lung Blood Flow That Could Determine Warfighter Success at High Altitude
Andrew Lovering (USA)

PROGRAM

EB 2014

Experimental Biology

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The American Physiological Society (APS)

American Society for Biochemistry and Molecular Biology (ASBMB)

American Society for Investigative Pathology (ASIP)

American Society for Nutrition (ASN)

American Society for Pharmacology and Experimental Therapeutics (ASPET)

Experimental Biology

April 26-30, 2014 – San Diego Convention Center

Physiology

APS President's Symposium Series

Multiscale Physiology: Linking Cellular and Molecular Insights to the Health of Organisms and Populations

428. PHYSIOLOGICAL RELEVANCE OF THE INTESTINAL MICROBIOME: MOVING BEYOND THE GUT

Symposium

(*Sponsored by:* President's Symposium Series)

Tue. 3:15 pm—San Diego Convention Center, Room 20A

Chaired: P. Lund

Gut Interactions

3:15 Overuse of antibiotics and the risk of obesity and asthma. **M.J. Blaser.** NYU Sch. of Med.

3:50 Not all in your mind — crosstalk between the microbiota and behavior. **M. Gareau.** UCSD.

4:25 Manipulating the microbiota to improve metabolic dysfunction. **H. Tilg.** Med. Univ. Innsbruck.

5:00 Panel discussion.

429. ALTITUDEOMICS 2012

Featured Topic

(*Sponsored by:* Environmental and Exercise Physiology Section)

Tue. 8:00 am—San Diego Convention Center, Room 28A

Chaired: R. Roach

Hypoxia

8:00 AltitudeOmics study overview: an approach to understanding human adaptation to high altitude. **B. Kayser.** Univ. of Geneva.

8:15 AltitudeOmics: the effect of high altitude ascent and acclimatisation on cerebral blood flow regulation **J-L. Fan, A.W. Subudhi, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, R.B. Panerai, A.T. Lovering and R.C. Roach.** Univ. of Lausanne, Univ. of Colorado Denver, Univ. of Colorado Colorado Springs, Univ. of Leicester and Univ. of Oregon. **(885.1)**

8:30 AltitudeOmics: pulmonary gas exchange efficiency in humans with and without a PFO following 16 days of acclimatization to 5,260 m **J. Elliott, S. Laurie, R. Goodman, K. Beasley, J. Kern, A. Subudhi, R. Roach and A. Lovering.** Univ. of Oregon, Univ. of Colorado Denver, Aurora and Univ. of Colorado, Colorado Springs. **(885.2)**

8:45 Oxygen tension regulates H_2S and NO bioavailability in reciprocal manner **C. Kevil, X. Shen, S. Shiva, M. Gladwin and R. Roach.** LSU Hlth. Sci. Ctr., Shreveport, Univ of Pittsburgh and Univ of Colorado Anshutz Med. Campus. **(885.3)**

9:00 Epigenetic modification of gene expression during human acclimatization to hypobaric hypoxia **C. Julian, A. Subudhi, O. Evero, B. Pedersen, D. Dvorkin, A. Lovering and R. Roach.** Univ. of Colorado Denver Anschutz Med. Campus and Univ. of Oregon. **(885.4)**

9:15 AltitudeOmics: hemoglobin mass and blood volume changes during early acclimatization to and de-acclimatization from 5260m **B.J. Ryan, N.B. Wachsmuth, W.F. Schmidt, W.C. Byrnes, A.T. Lovering, A.W. Subudhi and R.C. Roach.** Univ. of Colorado Boulder, Univ. of Bayreuth, Germany, Univ. of Oregon and Univ. of Colorado Denver, Aurora. **(885.5)**

9:30 Adenosine signaling via A2B receptor regulates erythrocyte 2,3-bisphosphate induction at high altitude **H. Liu, Y. Zhang, K. Sun, A. Song, H.K. Quintana, A. Grenz, R. Kellems, H. Eltzschig, R. Roach, M. Blackburn and Y. Xia.** Univ. of Texas Med. Sch., Univ. of Colorado Sch. of Med. and Univ. of Texas Med. Sch. at Houston. **(885.6)**

9:45 AltitudeOmics: physiologic and omic routes to high altitude adaptation. **R. Roach.** Univ. of Colorado Denver.

430. CELLULAR ADAPTATION AND SURVIVAL TO HYPOXIC CONDITIONS: EPIGENETIC MECHANISMS

Symposium

(*Sponsored by:* Physiological Genomics Group)

Tue. 8:00 am—San Diego Convention Center, Room 25A

Chaired: H. G. Klemcke

Cochaired: Y. Wang

Genetics

8:00 An introduction to epigenetic mechanisms: an evolving saga. **K. Susztak.** Albert Einstein Col. of Med.

8:30 Tolerance to brain ischemia: a role for epigenetics. **R. Meller.** Morehouse Sch. of Med.

9:00 Fetal epigenetic responses to hypoxia. **L. Zhang.** Loma Linda Univ.

9:30 Epigenetically-modified endothelial progenitor cells improve murine cardiac responses to infarction. **R. Kishore.** Feinberg Sch. of Med., Northwestern Univ.

LAST DAY TO VISIT EXHIBITS

Tuesday, April 29
9:00 AM – 4:00 PM

A351 **884.8** Endurance training ameliorates insulin resistance via suppressing of muscle endoplasmic reticulum stress **Y. Kadota, T. Sasaki, Y. Kitaura and Y. Shimomura.** Nagoya Univ.

A352 **884.9** Exercise training combined with insulin attenuates cardiac mitochondrial dysfunctions in diabetic rats **D.N. da Cunha, M.F. da Silva, A.J. Natali, E. da Silva, B.G. Teodoro, L.R. Drummond, F.R. Drummond, A.G. Moura, F.G. Belfort, A. Oliveira, T.N. Prímola-Gomes, I.R. Maldonado and L.C. Alberici.** Fed. Univ. of Viçosa, Fed. Univ. of Jequitinhonha and Mucuri Valleys and São Paulo State Univ., Brazil.

A353 **884.10** Influence of five days of reduced daily physical activity on cardiac baroreflex sensitivity during acute hyperglycemia **S.W. Holwerda, L.J. Boyle, D.P. Credeur, J.P. Thyfault and P.J. Fadel.** Univ. of Missouri-Columbia.

A354 **884.11** Physical exercise benefits on insulin sensitivity, vascular function and non-alcoholic fat liver disease in cafeteria diet-fed rats **C. de Moraes, L.K. Oharomari, A.G. Joaquim, N.F. Garcia, P.P. Ovidio and A.A. Jordão Junior.** Univ. of São Paulo, Ribeirão Preto.

A355 **884.12** Effect of 6-weeks voluntary wheel running on the sex-hormone and cytochrome P450 aromatase of high-fat feeding impuberism male SD rats **Y. Yan, M. Xie, J. Sun and Y. Zhao.** Beijing Sport Univ.

A356 **884.13** Systemic blood pressure predicts disease severity of idiopathic pulmonary arterial hypertension **X-G. Sun.** Natl. Ctr. of Cardiovasc. Dis., Fuwai Hosp., Beijing.

A357 **884.14** A single bout of moderate intensity resistance exercise improves neural mechanisms of arterial pressure control in L-NAME hypertensive rats **A.S. Barreto, M.M. Mota, T.L.T.B. da Silva, M.T. Fontes, V.J. Santana-Filho and M.R.V. Santos.** Fed. Univ. of Sergipe, Brazil.

A358 **884.15** Voluntary wheel running improves cardiac function in a rodent model of chronic kidney disease **J.M. Kuczmarski, C.R. Martens, J. Kim, S. Lennon-Edwards and D.G. Edwards.** Univ. of Delaware.

A359 **884.16** Moderate aerobic exercise training recovers disorders in white skeletal muscle induced by chronic kidney disease in rats **W.A. de Moraes, P.R. Souza, N.A. Paixao, L.H. Bozi, F.D. Guimaraes, M.C. Irigoyen, P.C. Brum and A. Medeiros.** Fed. Univ. of São Paulo, Heart Inst., São Paulo and Univ. of São Paulo.

A360 **884.17** Dietary quercetin enrichment improves respiratory function in mdx mice **J.T. Selsby, C. Ballman and J.C. Quindry.** Iowa State Univ. and Auburn Univ.

A361 **884.18** PGC-1α gene transfer rescues dystrophic muscle from advanced disease progression **K. Hollinger, E.R. Barton and J.T. Selsby.** Iowa State Univ. and Univ. of Pennsylvania.

A362 **884.19** Distinguishing differences between intrinsic aerobic capacity and age: a 1H-NMR metabolomics approach **J. Shearer, O.S. Falegan, R.T. Hepple, D.S. Hittel, L.G. Koch, S.L. Britton and H.J. Vogel.** Univ. of Calgary, Canada, McGill Univ. and Univ. of Michigan.

A363 **884.20** Metabolic profile and inflammatory markers in an endurance trained and untrained population **D.L. Vera, M. Castillo, K. Oliver, E. Rosario, M. Granquist and S.L. Dunn.** Univ. of La Verne, California Baptist Univ., California State Univ., Los Angeles and Casa Colina Hosp. for Rehabil. Med., Pomona.

A364 **884.21** The effect of cachectic myofiber metabolic properties on the resistance exercise-induced hypertrophy response **J.E. Mangum, J.P. Hardee, M.J. Puppa and J.A. Carson.** Univ. of South Carolina.

A365 **884.22** Exercise tolerance in Gulf War veterans and relationship to deployment exposures **W.A. Smith, J.C. Klein, D. Ndirangu, Y. Chen and M.J. Falvo.** Univ. of Memphis and VA New Jersey Hlth. Care Syst.

A366 **884.23** Temporal changes in left ventricular mechanics: impact of bed rest and exercise **J. Scott, M. Downs and L. Ploutz-Snyder.** NASA Johnson Space Ctr.

A367 **884.24** Weight loss with or without exercise: effect on classic and novel biomarkers of cardiovascular risk **C.R. Mikus, K.M. Huffman, L.M. Redman, E. Ravussin and W.E. Kraus.** Duke Univ. Med. Ctr. and Pennington Biomed. Res. Ctr., Baton Rouge.

A368 **884.25** Twelve weeks of community exercise improves health parameters in women living in a semi-rural township in South Africa **N.E. Brooks, J. Bowes, L. Gava, N. January, A. Esterhuizen and K.H. Myburgh.** Univ. of Stirling, U.K. and Univ. of Stellenbosch, South Africa.

A369 **884.26** Aerobic fitness and limiting factors of maximal performance in chronic low back pain patients **I.L. Duque, I.M. Urrutia and A. Duvallet.** Univ. de Caldas, Colombia, Univ. del Cauca Fac. of Hlth. Sci., Colombia and AP-HP Avicenne, Sorbonne Paris Cité, Bobigny.

A370 **884.27** Development of protocols to estimate maximal oxygen consumption and body composition in individuals with spinal cord injury **D. Terson de Paleville, D. Lorenz, J.P. McCulloch, S. Aslan, M. Kloby, M. Love, A. Walden and S. Harkema.** Univ. of Louisville.

A371 **884.28** Game time environmental conditions and concussion rate in college football **J.C. Harwood, A. Pennetti and K.J. Milne.** Univ. of Windsor, Canada.

A372 **884.29** Effects of treadmill exercise on spatial learning memory and upregulation of reelin signaling pathway in autistic rats **S-S. Baek, E-S. Ji and M-H. Lee.** Sangmyung Univ., South Korea.

885. NEW ADVANCES IN HUMAN ACCLIMATIZATION TO HYPOXIA

Poster

Mon. 7:30 AM—San Diego Convention Center, Exhibit Halls A-D

Presentation time: 12:45 PM–3:00 PM

A373 **885.1** AltitudeOmics: the effect of high altitude ascent and acclimatisation on cerebral blood flow regulation **J-L. Fan, A.W. Subudhi, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, R.B. Panerai, A.T. Lovering and R.C. Roach.** Univ. of Lausanne, Univ. of Colorado Denver, Univ. of Colorado Colorado Springs, Univ. of Leicester and Univ. of Oregon.

A374 **885.2** AltitudeOmics: pulmonary gas exchange efficiency in humans with and without a PFO following 16 days of acclimatization to 5,260 m **J. Elliott, S. Laurie, R. Goodman, K. Beasley, J. Kern, A. Subudhi, R. Roach and A. Lovering.** Univ. of Oregon, Univ. of Colorado Denver, Aurora and Univ. of Colorado, Colorado Springs.

A375 **885.3** Oxygen tension regulates H_2S and NO bioavailability in reciprocal manner **C. Kevil, X. Shen, S. Shiva, M. Gladwin and R. Roach.** LSU Hlth. Sci. Ctr., Shreveport, Univ of Pittsburgh and Univ of Colorado Anshutz Med. Campus.

M O N

A376 **885.4** Epigenetic modification of gene expression during human acclimatization to hypobaric hypoxia **C. Julian, A. Subudhi, O. Evero, B. Pedersen, D. Dvorkin, A. Lovering and R. Roach.** Univ. of Colorado Denver Anschutz Med. Campus and Univ. of Oregon.

A377 **885.5** AltitudeOmics: hemoglobin mass and blood volume changes during early acclimatization to and de-acclimatization from 5260m **B.J. Ryan, N.B. Wachsmuth, W.F. Schmidt, W.C. Byrnes, A.T. Lovering, A.W. Subudhi and R.C. Roach.** Univ. of Colorado Boulder, Univ. of Bayreuth, Germany, Univ. of Oregon and Univ. of Colorado Denver, Aurora.

A378 **885.6** Adenosine signaling via A2B receptor regulates erythrocyte 2,3-bisphosphate induction at high altitude **H. Liu, Y. Zhang, K. Sun, A. Song, H.K. Quintana, A. Grenz, R. Kellems, H. Eltzschig, R. Roach, M. Blackburn and Y. Xia.** Univ. of Texas Med. Sch., Univ. of Colorado Sch. of Med. and Univ. of Texas Med. Sch. at Houston.

886. PREGNANCY AND EXERCISE

Poster

MON. 7:30 AM—SAN DIEGO CONVENTION CENTER, EXHIBIT HALLS A-D

Presentation time: 12:45 PM–3:00 PM

A379 **886.1** Maternal exercise during pregnancy reduces risk of mammary tumorigenesis in rat offspring **W. Zheng, I. Camarillo, L. Clah, X. Zhou, B. Larrick, N. Blaize, E. Breslin, S.S. Donkin, T.P. Gavin, S. Newcomer and D. Teegarden.** Purdue Univ.

A380 **886.2** Effects of maternal exercise during pregnancy on coronary artery vascular function in high fat fed swine offspring **N. Blaize, E. Zartman, R. Cabot and S.C. Newcomer.** Purdue Univ. and California State Univ., San Marcos.

A381 **886.3** Influence of exercise mode on maternal and fetal health outcomes **C.M. Moyer and L.E. May.** East Carolina Univ.

A382 **886.4** Maternal exercise increases energy efficiency of fetal heart **L.E. May, D. Barrera, S. Newcomer and A.B. Agbas.** East Carolina Univ., Kansas City Univ. of Med. and Biosci. and California State Univ., San Marcos.

A383 **886.5** Influence of exercise in pregnancy on fetal heart response during labor and delivery **N.A. Barbari, L.E. May, E. Newton and J. Livingston.** East Carolina Univ.

A384 **886.6** Aerobic exercise in pregnant Latina women: effect on metabolic and body composition outcomes. A randomized clinical trial **R. Ramirez Velez.** Univ. Santo Tomas, Colombia.

A385 **886.7** Do the exercise habits of pregnant women conform to evidence-based recommendations? **A.L. McKenzie, L.A. Ellis, K. Fuller, I. Guelinckx, E.T. Perrier and L.E. Armstrong.** Univ. of Connecticut and Danone Res., Palaiseau.

887. HYPOXIA-INDUCED GENE EXPRESSION

Poster

MON. 7:30 AM—SAN DIEGO CONVENTION CENTER, EXHIBIT HALLS A-D

Presentation time: 12:45 PM–3:00 PM

A386 **887.1** Genome-wide detection of oxidized base products in guanine quadruplex (G4) sequences by ChIP-Seq analysis in hypoxic cells **V.M. Pastukh, G. Borchert and M.N. Gillespie.** Univ. of South Alabama Col. of Med. and Univ. of South Alabama.

A387 **887.2** Hypoxia reduces the function and expression of hERG potassium channels **S.M. Lamothe, W. Song, J. Guo and S. Zhang.** Queen's Univ., Canada.

A388 **887.3** Hypoxia-induced angiogenesis in the conditional HIF-1α deficient and HIF-2α deficient mice **K. Xu, X. Sun, C.P. Tsipis and J.C. LaManna.** Case Western Reserve Univ.

A389 **887.4** The purine nucleotide cycle: a cardioprotective pathway induced by HIF-1α **J. Wu, C. Bond, Y. Li and G. Wright.** East Tennessee State Univ.

A390 **887.5** Ketamine decreases immune gene expression in ovine fetal frontal cortex exposed to acute hypoxic hypoxia **E.I. Chang, M.B. Rabaglino, E. Richards and C.E. Wood.** Univ. of Florida Col. of Med., Univ. of Florida Col. of Agr. and Life Sci. and Univ. of Florida Col. of Pharm.

888. HYPOXIA/TRANSMITTERS, SECOND MESSENGERS, AND SIGNAL TRANSDUCTION

Poster

MON. 7:30 AM—SAN DIEGO CONVENTION CENTER, EXHIBIT HALLS A-D

Presentation time: 12:45 PM–3:00 PM

A391 **888.1** The effects of hypoxia in determining larval size in *Drosophila melanogaster* **D.M. Wong and J.A. Martinez-Agosto.** UCLA.

A392 **888.2** Effect of hypoxia on nuclear high affinity Ca^{2+} -ATPase activity and nuclear Ca^{2+} -influx in the cerebral cortex of newborn piglets **M. Delivoria-Papadopoulos, A. Wang, S. Malaeb and Q.M. Ashraf.** Drexel Univ. Col. of Med.

A393 **888.3** Hypoxia augments intracellular hydrogen sulfide in LPS-stimulated murine macrophages **E.R. DeLeon and K.R. Olson.** Indiana Univ. Sch. of Med.-South Bend and Univ. of Notre Dame.

A394 **888.4** Renal epithelial electrophysiological response to cobalt chloride induced hypoxia **S. Nag and A. Resnick.** Cleveland State Univ.

889. HYPOXIA AND ION CHANNELS

Poster

MON. 7:30 AM—SAN DIEGO CONVENTION CENTER, EXHIBIT HALLS A-D

Presentation time: 12:45 PM–3:00 PM

A395 **889.1** T-type calcium channels in carotid body oxygen sensing **V.V. Makarenko, Y-J. Peng, L. Piao, J. Nanduri and N.R. Prabhakar.** Univ. of Chicago.

A396 **889.2** The crucial role of calcium sensing receptor for hypoxia-induced pulmonary arterial hypertension in mice **H. Tang, S. Song, A.R. Drennan, R. Fernandez, J. Chen and J.X-J. Yuan.** Univ. of Illinois at Chicago.

EB 2014 San Diego
Experimental Biology
April 26-30, 2014 San Diego Convention Center

Presentation Abstract

Presentation Title: Adenosine signaling via A2B receptor regulates erythrocyte 2,3-bisphosphate induction at high altitude

Presentation Time: Tuesday, Apr 29, 2014, 9:30 AM - 9:45 AM

Speaker(s): Hong Liu[1], Yujin Zhang[1], Kaiqi Sun[1], Anren Song[1], Harry Karmouty Quintana[1], Almut Grenz[2], Rodney Kellems[1], Holger Eltzschig[3], Robert Roach[4], Michael Blackburn[1], **Yang Xia**[1]

[1]University of Texas-Medical School, Houston, TX,[2]University of Colorado School of Medicine, Denver, CO,[3]University of Colorado School of Medicine, Houston, TX,[4]University Colorado Denver, Denver, CO

H. Liu: None. **Y. Zhang:** None. **K. Sun:** None. **A. Song:** None. **H. Quintana:** None. **A. Grenz:** None. **R. Kellems:** None. **H. Eltzschig:** None. **R. Roach:** None. **M. Blackburn:** None. **Y. Xia:** None.

Sponsoring Society: Physiology - The American Physiological Society (APS) - Sponsoring Society

Topic: 1136-APS AltitudeOmics 2012 (Roach)

Abstract: To function effectively in O_2 uptake, and unload, erythrocytes rely on sophisticated regulation of hemoglobin (Hb)-O_2 affinity by allosteric modulators. One of the best known allosteric modulators is 2,3-bisphophosphoglycerate (2,3-BPG). Earlier studies demonstrated that erythrocyte 2,3-BPG levels are elevated at a high altitude. However, what triggers its induction at high altitude is unknown. To address this question, we recruited 24 individuals and placed them at high altitude for different time points. Here we report that 1) Plasma adenosine and erythrocyte 2,3-BPG levels were significantly elevated in normal individuals at high altitude after 24 hours compared to sea level; 2) their levels are further enhanced at high altitude after 16 days; 3) Elevated circulating adenosine levels significantly correlated with increased erythrocyte 2,3-DPG levels in normal individuals at high altitude. Next, we provide *in vitro* evidence that adenosine signaling via A2B receptor (ADORA2B) directly induced 2,3-BPG production in a protein kinase A-dependent manner in cultured human erythrocytes. Finally, we provide genetic and pharmacological evidence that that ADORA2B is essential for elevated adenosine induced 2,3-BPG production and triggers O_2 release in mice. Taken together, we reveal ADORA2B signaling cascade regulating erythrocyte 2,3-BPG induction as a function of altitude and identify new targets to enhance O_2 release under hypoxia conditions.

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EB 2014 Experimental Biology San Diego April 26-30, 2014 San Diego Convention Center

Presentation Abstract

Presentation Title:	AltitudeOmics: hemoglobin mass and blood volume changes during early acclimatization to and de-acclimatization from 5260m
Presentation Time:	Tuesday, Apr 29, 2014, 9:15 AM - 9:30 AM
Speaker(s):	**Benjamin J. Ryan**[1], Nadine B. Wachsmuth[2], Walter F. Schmidt[2], William C. Byrnes[1], Andrew T. Lovering[3], Andrew W. Subudhi[4], Robert C. Roach[4] [1]Integrative Physiology, University of Colorado, Boulder, CO,[2]Sports Medicine/Physiology, University of Bayreuth, Bayreuth, Germany,[3]Human Physiology, University of Oregon, Eugene, OR,[4]Altitude Research Center, University of Colorado Denver, Aurora, CO **B.J. Ryan:** None. **N.B. Wachsmuth:** None. **W.F. Schmidt:** None. **W.C. Byrnes:** None. **A.T. Lovering:** None. **A.W. Subudhi:** None. **R.C. Roach:** None.
Sponsoring Society:	Physiology - The American Physiological Society (APS) - Sponsoring Society
Topic:	1136-APS AltitudeOmics 2012 (Roach)
Abstract:	The early time course of changes in hemoglobin mass (Hbmass) and red cell volume (RCV) following ascent to and descent from altitudes greater than 5000m has not been previously determined in humans. We examined Hbmass and blood volume (BV) compartments in healthy men (n = 12) and women (n = 9) at sea level (SL) and 5260m following 1, 7, and 16 days of altitude exposure (ALT1/ALT7/ALT16). Subjects were also studied upon return to 5260m following descent to 1525m of either 7 or 21 days. Hbmass was assessed using the optimized CO rebreathing method. Compared to SL, absolute Hbmass was not different at ALT1 but increased by 3.7 ± 5.8% (n = 20; p < 0.01) at ALT7 and 7.6 ± 6.6% (n = 21; p < 0.001) at ALT16. Following descent to 1525m, Hbmass was reduced compared to ALT16 (-6.0 ± 3.7%; n = 20; p = 0.001) and not different from SL, with no difference in the loss in Hbmass between groups that descended for 7 vs. 21 days. There was a large correlation between the loss in Hbmass and increase in serum ferritin following 7 days at 1525m (r = -0.64; n = 13; p < 0.05), suggesting increased red blood cell destruction. We found significant reductions in absolute and relative plasma volume (PV) of ~9-12% at ALT7 and ALT16 compared to SL; PV returned to SL values following descent to 1525m. Relative BV was not different from SL at any time point due to opposing changes in PV and RCV. Our results demonstrate that large changes in Hbmass can occur within 1-2 weeks in lowlanders during acclimatization to and de-acclimatization from altitudes greater than 5000m.

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Presentation Abstract

Presentation Title:	Epigenetic modification of gene expression during human acclimatization to hypobaric hypoxia
Presentation Time:	Tuesday, Apr 29, 2014, 9:00 AM - 9:15 AM
Speaker(s):	**Colleen Julian**[1], Andrew Subudhi[1], Oghenero Evero[1], Brent Pedersen[1], Daniel Dvorkin[1], Andrew Lovering[2], Robert Roach[1] [1]University of Colorado Denver Anschutz Medical Campus, Aurora, CO,[2]University of Oregon, Eugene, OR **C. Julian:** None. **A. Subudhi:** None. **O. Evero:** None. **B. Pedersen:** None. **D. Dvorkin:** None. **A. Lovering:** None. **R. Roach:** None.
Sponsoring Society:	Physiology - The American Physiological Society (APS) - Sponsoring Society
Topic:	1136-APS AltitudeOmics 2012 (Roach)
Abstract:	Epigenetic processes and hypoxia-inducible transcription factors work in coordination to regulate transcriptional responses to hypoxia. Our aim was to determine the role of epigenetics and associated changes to gene transcription for human acclimatization to high altitude. We performed genome-wide methylation (Infinium HumanMethylation450 BeadChip, Illumina) and expression (Affymetrix Human Gene 1.0 ST Array) studies in peripheral blood mononuclear cells obtained from 21 healthy individuals at sea level and on three occasions during acclimatization to 5260m. Acute hypoxia minimally affected DNA methylation status. After 16 days of acclimatization, we identified 183 differentially methylated sites, including several that are associated with genes known to be involved in hypoxic response (e.g., eukaryotic translation initiation factor 2C subunit 2 [EIF2C], heat shock protein [HSP] 27, DNA (cytosine-5)-methyltransferase 3A). Indicating the potential functional importance of these epigenetic modifications, using methQTL we found that methylation status altered the transcriptional activity of hypoxia-related genes considered central to human acclimatization to hypoxia (e.g., HIF1AN, EIFs, HSPs). Our findings support the possibility that epigenetic modification of gene expression contributes to acclimatization to high altitude.

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EB 2014 San Diego
April 26-30, 2014 San Diego Convention Center

Presentation Abstract

Presentation Title:	AltitudeOmics: the effect of high altitude ascent and acclimatisation on cerebral blood flow regulation
Presentation Time:	Tuesday, Apr 29, 2014, 8:15 AM - 8:30 AM

Speaker(s): **Jui-Lin Fan**[1,2], Andrew W. Subudhi[3,4], Oghenero Evero[3], Nicolas Bourdillon[1], Bengt Kayser[1], Colleen G. Julian[3], Ronney B. Panerai[5], Andrew T. Lovering[6], Robert C. Roach[3]

[1]Institute of Sports Sciences, University of Lausanne, Lausanne, Switzerland,[2]Lemanic Neuroscience Doctoral School, University of Lausanne, Lausanne, Switzerland,[3]Altitude Research Center, University of Colorado, Denvor, CO,[4]Department of Biology, Colorado Spings, CO,[5]Leicester Royal Infirmary, University of Leicester, Leicester, United Kingdom,[6]Department of Human Physiology, University of Oregon, Eugene, OR

J. Fan: None. **A.W. Subudhi:** None. **O. Evero:** None. **N. Bourdillon:** None. **B. Kayser:** None. **C.G. Julian:** None. **R.B. Panerai:** None. **A.T. Lovering:** None. **R.C. Roach:** None.

Sponsoring Society: Physiology - The American Physiological Society (APS) - Sponsoring Society

Topic: 1136-APS AltitudeOmics 2012 (Roach)

Abstract: Adequate oxygen supply to the brain is critical to maintain brain function. Hypoxia presents a unique challenge in maintaining sufficient cerebral oxygen delivery (DO2). We assessed by ultrasound cerebral blood flow (CBF: internal carotid, vertebral arteries and middle cerebral artery velocity [MCAv]) and arterial blood pressure (index of cerebral autoregulation; CA) during rest and hypercapnic breathing (MCAv-CO2 slope; index of cerebrovascular function) in 21 healthy subjects at sea-level (SL) and upon arrival at 5260m (ALT1) and after 16 days of acclimatisation (ALT16). Cerebral DO2 was calculated as the product of arterial oxygen content (CaO2) and flow in each respective artery and summed to estimate global CBF. Global CBF increased ~70% upon arrival at ALT1 ($P<0.05$) and returned to SL values at ALT16 as a result of changes in cerebral vascular resistance. A reciprocal pattern in CaO2 maintained global cerebral DO2 across acclimatisation. MCAv-CO2 slope was elevated by ~79% upon arrival at ALT1 and further increased by ~89% at ALT16 ($P<0.05$). Indexes of CA were reduced upon arrival at ALT1 ($P<0.05$), but did not change with acclimatisation at ALT16 ($P>0.10$). Cerebral DO2 was well maintained upon acute exposure and acclimatisation to hypoxia. Cerebrovascular function was enhanced with acclimatisation to high altitude, but these changes did not mitigate the reduction in CA associated with hypoxic exposure.

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EB 2014 Experimental Biology San Diego
April 26-30, 2014 San Diego Convention Center

Presentation Abstract

Presentation Title:	AltitudeOmics: pulmonary gas exchange efficiency in humans with and without a PFO following 16 days of acclimatization to 5,260 m
Presentation Time:	Tuesday, Apr 29, 2014, 8:30 AM - 8:45 AM
Speaker(s):	**Jonathan Elliott**[1], Steven Laurie[1], Randall Goodman[1], Kara Beasley[1], Julia Kern[1], Andrew Subudhi[2,3], Robert Roach[3], Andrew Lovering[1] [1]Human Physiology, University of Oregon, Eugene, OR,[2]Biology, University of Colorado, Colorado Springs, CO,[3]Anschutz Medical Campus, University of Colorado, Aurora, CO **J. Elliott:** None. **S. Laurie:** None. **R. Goodman:** None. **K. Beasley:** None. **J. Kern:** None. **A. Subudhi:** None. **R. Roach:** None. **A. Lovering:** None.
Sponsoring Society:	Physiology - The American Physiological Society (APS) - Sponsoring Society
Topic:	1136-APS AltitudeOmics 2012 (Roach)
Abstract:	A PFO is a source of intracardiac shunt causing impaired pulmonary gas exchange efficiency, defined by an increased alveolar-to-arterial PO_2 difference ($AaDO_2$). Prior studies investigating human acclimatization to high altitude (HA) have not investigated differences between subjects with a patent foramen ovale (PFO+) and those without (PFO–), yet prevalence of PFO in the general population is ~40%. Twenty-one (11 PFO+) healthy lowlanders were studied at rest and at 70, 100, 130, and 160W of cycle ergometer exercise at sea level (SL), in acute hypoxia at 5,260 m (ALT1), and after 16 days of acclimatization to 5,260 m (ALT16). Exercise data were compared at the highest iso-workload, within an individual, achieved at SL, ALT1 and ALT16. During exercise at SL, PFO+ subjects demonstrated a wider $AaDO_2$ compared to PFO– subjects, however on ALT1 the $AaDO_2$ was not different between PFO– and PFO+ subjects. At ALT16, unlike PFO– subjects, $AaDO_2$ in PFO+ subjects was not different from ALT1. Surprisingly, at ALT16 the PFO+ group did not demonstrate an increase in resting minute ventilation and consequently, did not increase either alveolar PO_2 or arterial PO_2 relative to ALT1. Taken together, our data suggest that 1) intracardiac shunt in PFO+ subjects results in significantly worse pulmonary gas exchange efficiency after acclimatization to HA and 2) these subjects demonstrate physiological changes consistent with a reduced ability to acclimatize to HA.

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EB 2014 Experimental Biology San Diego April 26-30, 2014 San Diego Convention Center

Presentation Abstract

Presentation Title:	Oxygen tension regulates H_2S and NO bioavailability in reciprocal manner
Presentation Time:	Tuesday, Apr 29, 2014, 8:45 AM - 9:00 AM
Speaker(s):	**Christopher Kevil**[1], Xinggui Shen[1], Shruti Shiva[2], Mark Gladwin[3], Robert Roach[4]
	[1]Pathology, LSU Health Sciences Center, Shreveport, LA,[2]Pharmacology and Chemical Biology, Univ of Pittsburgh, Pittsburgh, PA,[3]Pulmonary, Allergy and Critical Care Medicine, Univ of Pittsburgh, Pittsburgh, PA,[4]Emergency Medicine, Univ of Colorado Anshutz Medical Campus, Aurora, CO
	C. Kevil: None. **X. Shen:** None. **S. Shiva:** None. **M. Gladwin:** None. **R. Roach:** None.
Sponsoring Society:	Physiology - The American Physiological Society (APS) - Sponsoring Society
Topic:	1136-APS AltitudeOmics 2012 (Roach)
Abstract:	Nitric oxide and hydrogen sulfide modulate several physiological and cellular functions including vasodilation, cell proliferation and redox cell signaling. Production and bioavailability of either molecule may be influenced by oxygen tension; however, the relationship between H2S and NO bioavailability during hypoxia remains poorly understood. In this study, we examined plasma free H2S and nitrite levels in healthy volunteers exposed to high altitude. Plasma free H2S levels were significantly greater at sea level compared to high altitude (5260 meters) at 1 or 16 days; whereas, plasma nitrite levels were significantly higher at high altitude compared to sea level. After spending 7 or 21 days at low altitude, volunteers were tested again at 5260 m. On reascent, plasma free H2S levels were increased compared to initial high altitude exposure. Conversely, plasma nitrite levels were lower than high altitude exposure on reascent indicating reciprocal regulation between the gasotransmitters. Calculation of plasma free H2S to nitrite ratios revealed that high altitude acclimatization decreases the ratio of plasma free H2S to nitrite, and that on reascent H2S to nitrite ratios return to low altitude levels. In vitro cellular hypoxia studies using HUVEC revealed significant decreases in acid labile sulfide while increasing protein bound biochemical forms of sulfide. Interestingly, in vitro hypoxic challenge similarly decreased free H2S to nitrite ratios in HUVEC similar to high altitude exposure. These data demonstrate a unique reciprocal relationship between H2S and NO bioavailability during hypoxia.

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ATS 2014

"IMPACT OF HYPOXIA ON CARDIO-METABOLIC HEALTH: GOOD OR EVIL?"

The session will be held on Sunday, May 18, 8:15 to 10:45 AM.

1. Is hypoxia the root of evil in sleep apnea? Basic science perspective

8:15-8:40 Dr. Vsevolod Polotsky, MD PhD. Johns Hopkins Univ School of Medicine

2. Is hypoxia the root of evil in sleep apnea? Clinical perspective

8:40-9:10 Dr. Daniel Gottlieb, MD, MPH. Boston Univ School of Medicine

3. Can hypoxia improve cardio-metabolic health? Basic science perspective

9:10-9:35 Dr. Christopher O'Donnell, Ph.D. University of Pittsburgh School of Medicine

4. Can hypoxia improve cardio-metabolic health? Clinical perspective

9:35-10:05 Dr. Richard Mackenzie, PhD. University of Westminster, Human and Health Services UK

5. Evils and benefits of hypoxia: Lessons from altitude and athletics

10:05-10:35 Dr. Robert Roach, PhD. University of Colorado Altitude Research Center.

6. Summary

10:35-10:45 Jonathan Jun, MD. Johns Hopkins Univ School of Medicine

CONFERENCE PROGRAM

International Conference on Systems Biology 2014
14–18 September 2014

ICSB 2014
The International Systems Biology Conference
MELBOURNE AUSTRALIA
14–18 SEPTEMBER 2014

Sunday 14 September 2014		
0900–1800	COMBINE/ERAsysAPP Tutorial: Modeling and simulation of biological models view full outline	Ether Conference Centre, On15
1500–1800	Registration open	Melbourne Convention Centre foyer
1700	Delegates at leisure	Drink vouchers provided for South Wharf Promenade

Monday 15 September 2014		
0700–1900	Registration open	Melbourne Convention Centre foyer
0830–0845	Opening ceremony	Plenary three
0845–1030	**Health and wellbeing**	Plenary three
Chairperson	**Prof Nadia Rosenthal**, *Australian Regenerative Medicine Institute*	
0845–0915	Systems Biology of Stem Cells, **Prof Huck Hui Ng,** *Genome Institute of Singapore*	**Sponsored by Stem Cells Australia** STEMCELLS AUSTRALIA
0915–0945	The routes of reprogramming to alternative states of pluripotency, **Dr Andres Nagy**, *Mount Sinai Hospital*	**Sponsored by SBI Australia** SBI The Systems Biology Institute AUSTRALIA
0945–1015	Systems medicine for novel avenues into cancer diagnostics and therapy, **Prof Roland Eils**, *Heidelberg University*	
1015–1030	Identification of common disrupted protein networks in metastasis across multiple cancer types, **Dr Melissa Davis**, *University of Melbourne*	

Correct as of Friday, September 12, 2014

AltitudeOmics: Integrating physiology and OMICS to understand human acclimatization to hypoxia

Andrew W. Subudhi, Nicolas Bourdillon, Daniel Dvorkin, Jonathan E. Elliott, Oghenero Evero, Jui-Lin Fan, Jeff Groenwold, Sonja Jameson-Van Houten, Colleen G. Julian, Bengt Kayser, Julia P. Kern, Steven S. Laurie, Andrew T. Lovering and Robert C. Roach

Altitude Research Center, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; Institute of Sports Sciences and Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, SWITZERLAND; and the Department of Human Physiology, University of Oregon, Eugene, Oregon, USA.

Background: Understanding the human response to hypoxia is important for the health of millions of people worldwide who visit, live, or work in the hypoxic environment encountered at high altitudes. The basic mechanisms controlling acclimatization to hypoxia remain largely unknown. The AltitudeOmics project aimed to bridge this gap. Our goals were 1) to describe a phenotype for successful human adjustment to hypoxia and assess its retention and 2) use these findings as a foundation for companion OMICS-based mechanistic studies (transcriptomics and epigenetics).

Methods: We report physiological and OMICS findings from 21 subjects as they acclimatized to hypoxia at 5260 m over 16 days; and when they reascended to 5260 m after either 7 (n=14) or 21 (n=7) days at 1525 m.

Results: At 16 days we observed: 1) increases in arterial oxygenation and [Hb] (compared to acute hypoxia: PaO_2 rose 9 ± 4 mmHg to 45±4 while $PaCO_2$ dropped a further 6±3 mmHg to 21±3, and [Hb] rose 1.8±0.7 g/dL to 16±2 g/dL; 2) no AMS; 3) improved cognitive function; and 4) improved exercise performance by 8±8% (all changes p<0.01). Upon reascent, retention was noted for arterial oxygenation but not [Hb], protection from AMS, retention of exercise performance, less retention of cognitive function. Marked changes were observed in gene expression and its epigenetic regulation during the onset and retention of acclimatization.

Conclusion: This discovery-based study suggests several novel avenues for future studies focused on discovery of the mechanisms underlying human acclimatization to hypoxia.

Keywords: hypoxia, transcriptomics, epigenetics

Leh Symposium 2014

"Ventilation and Circulation in Hypoxia: from mechanisms to patients and back"

(19th-23rd SEPTEMBER, 2014)

anisms to patients and back " The third international "Leh Symposiu

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Scientific Program

September 19, 2014, Friday	
Arrival, Leh (3500m)	Early morning
Rest (for acclimatization to high-altitude)	Up to 15:00 hrs
Scientific committee meeting Registration (parallel)	15:00 – 16:30 hrs 15:00 – 17:00 hrs
Inauguration & scientific session Dinner	17:00 – 19:30 hrs 20:00 hrs onward
September 20, 2014, Saturday	
Scientific Session I: Acclimatization, adaptation and maladaptation to high altitude	09:00 – 11:00 hrs
Tea time	11:00 – 11:25 hrs
Scientific Session II: Erythropoietin in acclimatization and (mal) adaptation (EpoCan)	11:30 – 13:10 hrs
Lunch	13:10 – 14:00 hrs

IGIB Institute of Genomics & Integrative Biology Delhi, India

S.N.M.Hospital Leh Ladakh J& K, India

PVRI Pulmonary Vascular Research Institute UK/USA

Abstract for 3rd Leh Conference
Ventilation and Circulation in Hypoxia

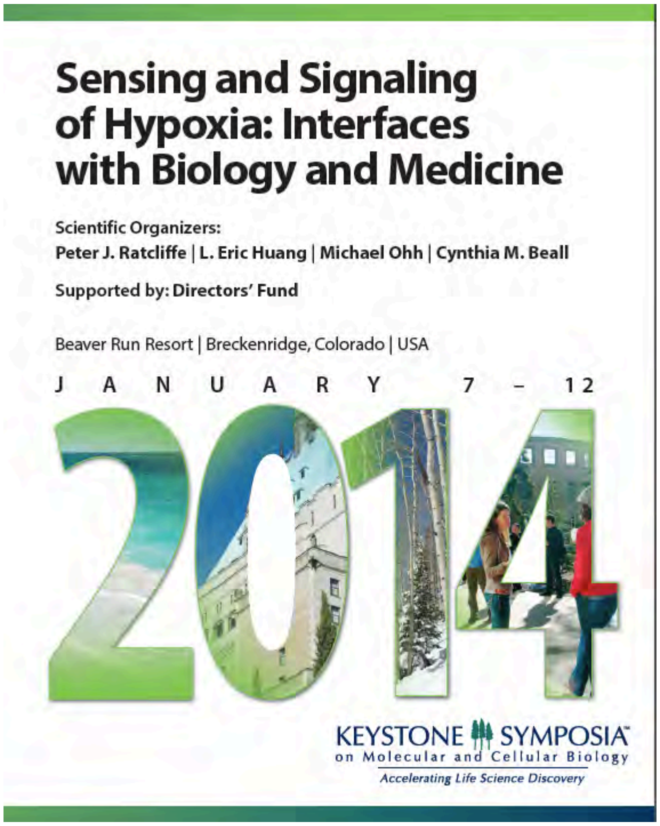
AltitudeOmics: Integrating physiology and OMICS to understand human acclimatization to hypoxia.

Andrew W. Subudhi[1], Nicolas Bourdillon[2], Jonathan E. Elliott[3], Oghenero Evero[1], Jui-Lin Fan[2], Sonja Jameson-Van Houten[1], Colleen G. Julian1, Bengt Kayser[2], Julia P. Kern[2], Steven S. Laurie[2], Andrew T. Lovering[2] and Robert C. Roach[1].

[1]Altitude Research Center, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; [2]Institute of Sports Sciences and Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland; and the [3]Department of Human Physiology, University of Oregon, Eugene, Oregon, USA.

An understanding of human responses to hypoxia is important for the health of millions of people worldwide who visit, live, or work in the hypoxic environment encountered at high altitudes. In spite of dozens of studies over the last 100 years, the basic mechanisms controlling acclimatization to hypoxia remain largely unknown. The AltitudeOmics project aimed to bridge this gap. Our goals were 1) to describe a phenotype for successful acclimatization and assess its retention and 2) use these findings as a foundation for companion OMICS-based mechanistic studies (transcriptomics and epigenetics). We report physiological and OMICS findings from 21 subjects as they acclimatized to 5260 m over 16 days; and when they reascended to 5260 m after either 7 (n=14) or 21 (n=7) days at 1525 m. At 16 days at 5260 m we observed: 1) increases in arterial oxygenation and [Hb] (compared to acute hypoxia: PaO2 rose 9 ± 4 mmHg to 45 ± 4 while PaCO2 dropped a further 6 ± 3 mmHg to 21 ± 3, and [Hb] rose 1.8 ± 0.7 g/dL to 16 ± 2 g/dL; 2) no AMS; 3) improved cognitive function; and 4) improved exercise performance by 8 ± 8% (all changes $p < 0.01$). Upon reascent, we observed retention of arterial oxygenation but not [Hb], protection from AMS, retention of exercise performance, less retention of cognitive function; and noted that some of these effects lasted for 21 days. Marked changes were observed in gene expression and its epigenetic regulation during the onset and retention of acclimatization. This discovery-based study suggests several novel avenues for future studies focused on discovery of the mechanisms underlying human acclimatization to hypoxia.

Grant support: The overall AltitudeOmics study was funded, in part, by grants from the U.S. Department of Defense (W81XWH-11-2-0040 TATRC to RR, and W81XWH-10-2-0114 to ATL). The project was also supported, in part, by NIH/NCATS Colorado CTSI Grant Number UL1 TR000154.



Poster Session 2: Thursday, January 9

2013 Convergent evolution of hypoxia adaptation in laboratory selected *Drosophila melanogaster* and in high altitude human populations

Aashish R. Jha[1,2], Christopher D. Brown[1,2], Dan Zhou[3,4], Gabriel G. Haddad[3,4], Martin Kreitman[1,5] and Kevin P. White[1,2,5]
[1]Institute for Genomics and Systems Biology; [2]Department of Human Genetics, [5]Department of Ecology and Evolution, University of Chicago, USA; [3]Department of Pediatrics, Division of Respiratory Medicine, University of California at San Diego, USA; [4]Rady Children's Hospital, San Diego, CA, USA

The ability to withstand low oxygen (hypoxia) is a highly polygenic yet mechanistically conserved trait that has important implications for both human health and evolution. However, genetic mechanisms involved in hypoxia adaptation in high altitude human populations remain elusive. We used experimental evolution followed by whole-genome sequencing in *Drosophila melanogaster* to investigate the role of natural variation in adaptation to hypoxia. Using a Generalized Linear Model we identified significant allele frequency divergences between three independently evolved hypoxia-tolerant populations and normoxic controls for ~3,800 single nucleotide polymorphisms. Over 1,600 of these variants, distributed throughout the genome, occur at evolutionary conserved positions and 155 genes harboring them are differentially expressed between hypoxia tolerant and normoxic populations. Comparison of our results with previous genome-wide studies in Tibetan, Andean, and Ethiopian high-altitude human populations revealed statistically significant overlap of shared positively selected genes. Of the 66 orthologous genes shared between the hypoxia adapted flies and high altitude humans, 12 genes such as — *AQP1* (*CG7777*), *ARNT* (*tgo*), *ACE* (*Ance-3*), *ELF2* (*EIP74EF*), *NR4A2* (*Hr38*), *CHRNB2* (*gfA*) and *HES1* (*hairy*) — have known functions in hypoxia in humans.

Our results show reproducible, convergent evolution among experimentally selected populations of *Drosophila* and the significant overlap of known hypoxia genes with high altitude humans suggests that fundamental genetic mechanisms regulating hypoxia-tolerance has remained conserved throughout evolution.

2014 Genome-wide DNA methylation patterns are altered during human acclimatization to hypobaric hypoxia

CG Julian[1,2], AW Subudhi[1,3], O Evero[1], BD Pedersen[2], D Dvorkin[1], AT Lovering[4], RC Roach[1]
[1]Altitude Research Center, Department of Emergency Medicine and [2]Department of Medicine, University of Colorado Denver, Aurora, CO; [3]Department of Biology, University of Colorado Colorado Springs, Colorado Springs, CO; [4]Department of Human Physiology, University of Oregon, Eugene, OR.

Epigenetic processes and hypoxia-inducible transcription factors work in coordination to regulate transcriptional responses to hypoxia. To identify whether epigenetic modifications are important for human acclimatization to high altitude and the retention of an acclimatized phenotype we performed genome-wide methylation studies (Infinium HumanMethylation450 BeadChip, Illumina) of peripheral blood mononuclear cells obtained from 21 healthy individuals at sea level, after acclimatization to 5260m, and upon re-exposure after 7 days of de-acclimatization at low altitude. Acute hypoxia minimally affected DNA methylation status. After 16 days of acclimatization, we identified 183 differentially methylated sites (139 mapped to genes), including several within genes known to be involved in hypoxic response (e.g., eukaryotic translation initiation factor 2C subunit 2 [EIF2C], heat shock protein [HSP] 27, DNA (cytosine-5)-methyltransferase 3A). Indicating the potential functional importance of these epigenetic modifications, the expression of epigenetically-modified genes also differed during acclimatization (e.g., EIFs, HSPs, adaptor-associated kinase 1 [AAK1]). Supporting the possibility that epigenetic mechanisms are involved in the retention of acclimatization, only one methylation change acquired during acclimatization was lost after 7 days at low altitude. Human acclimatization to high altitude is paralleled by epigenetic modifications that influence the expression of genes with known relationships to hypoxic response. Further study of the functional effects of such epigenetic events will improve our understanding of the mechanisms responsible for human acclimatization to hypoxia.

Grant support: This study was supported by the U.S. Department of Defense (W81XWH-11-2-0040 TATRC to RR). The project was also supported, in part, by NIH/NCATS Colorado CTSI Grant Number UL1 TR000154. Contents are the author's sole responsibility and do not necessarily represent official NIH views.
Dr. Julian is supported by a NIH Building Interdisciplinary Research Careers Women's Health (5 K12 HD057022-07).

15 May 2014

PRE-PROPOSAL FOR

Three New Ideas to Protect Special Forces from the Stress of High Altitude

Research Areas of Interest

3. Force Health Protection and Environmental Medicine
a. Optimal Acclimatization Strategy
b. High Altitude Pulmonary Edema/High Altitude Cerebral Edema

Prepared By

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Keywords: high-altitude, AMS, HAPE, HACE, acclimatization, edema, hypoxia, nifedipine, methazolamide, quercetin, metformin, nutraceutical

1. Scope/Introduction. (limit: 20 lines of text)

High altitude illnesses pose a significant threat to the warfighter rapidly exposed to high altitudes.[1] Unfortunately, no major advances have been made in promoting acclimatization or preventing high-altitude illnesses in the last 25 years. **We propose to test three novel ideas to rapidly advance warfighter performance in a state-of-the-art field study.**

In other recent DoD-funded studies we have been very productive. We have completed the first ever test of a gene-based prediction of high-altitude illness, and we have completed the first molecular and cellular biology study of how humans adjust to hypoxia.[2] Additionally, our colleagues have completed DARPA-funded work to screen hundreds of compounds for effectiveness in preventing high altitude pulmonary and cerebral edema. Each of these studies contributes to the novel ideas presented here. **Thus, our team is currently the best-suited civilian or military team in the world to address the scientific medical concerns of US Special Forces related to high altitude.**

To improve high-altitude performance we will test: **quercetin, nifedipine + methazolamide and metformin.** Quercetin, an over-the-counter nutraceutical, and nifedipine+methazlamide, two drugs already approved for use in humans, are effective for preventing high-altitude cerebral and pulmonary edema. Metformin, a drug commonly used to treat diabetes, induces biochemical changes recently linked to successful acclimatization and protection from high altitude illnesses. **We propose one or more of these approaches will substantially improve warfighter performance in the high altitude environment.**

2. Background Information and Scientific Approach. (limit: 2 pages of text)

This proposal directly addresses: *3. Force Health Protection and Environmental Medicine: a. Optimal Acclimatization Strategy; and b. High Altitude Pulmonary Edema/High Altitude Cerebral Edema*.

SOCCOM understands the importance of protecting SOF warfighters from the challenges of high-altitude illness, so the rationale for these experiments is brief. Acute mountain sickness (AMS) can cause debilitating symptoms—the PI has personally observed a Marine Corps Force Recon operator give up command of his unit on a training exercise due to symptoms of AMS incurred on rapid insertion to 14,000 ft. High-altitude pulmonary edema (HAPE) and high-altitude cerebral edema (HACE) can kill.[1] Although HAPE/HACE incidence is low, their impact can be great. For example, if a warfighter develops HAPE or HACE, immediate evacuation may be necessary. Such a diversion raises potential catastrophic risks for other troops. In support of the cost and risk of high-altitude illnesses is that during a major combat operation in Afghanistan, ~12% of medevacs and hospital admissions were due to severe AMS/HAPE/HACE. **This proposal directly addresses the need of SOF warfighters to expand their arsenal of options when working at high altitudes.**

We understand SOCCOM is interested in solutions, today. Therefore we have devised an experiment to test three different compounds for advancing acclimatization and preventing high-altitude illnesses. **Each can be tested today. If successful, any of the three could be used by SOF warfighters tomorrow.**

Next we outline why each of these three compounds shows promise for improving performance and preventing high-altitude illnesses. Then we briefly describe our experimental approach.

What is quercetin and why might it work to aid acclimatization and prevent high-altitude illnesses? Quercetin is an antioxidant and an anti-inflammatory agent, widely present in fruits and vegetables.[3] It has been reported to possess antioxidant effects as free radical scavenger, hydrogen-donating compound, a singlet oxygen quencher, and metaloid chelator. Quercetin can also reduce inflammation by attenuation of the redox-sensitive transcription factor and of scavenging free radicals. **We think quercetin will be effective for preventing high-altitude illnesses because 1) animal studies have shown it to be very effective in preventing cerebral edema,[4,5] and 2) it acts by suppressing inflammation.** We have recently shown in human that lower levels of inflammation are associated with protection from acute mountain sickness.[6] And dexamethasone, a potent anti-inflammatory steroid, is the most potent drug known for preventing and treating AMS, HAPE and HACE. In quercetin treated rats, each dose of quercetin reduced brain water content and transvascular leakage to near normoxia values, comparable to the protection afforded by dexamethasone. Although dexamethasone is extremely effective in preventing and treating high-altitude illnesses, its safety profile is troublesome. Thus, an alternative to dexamethasone is desirable. We think quercetin fits the role for a potential dexamethasone replacement, and it is attractive as a safe, over-the-counter medication that could be used by SOF warfighters immediately.

What is nifedipine+methazolamide, and why might it work to prevent high-altitude illnesses? Nifedipine is a proven to prevent HAPE by lowering pulmonary artery pressure.[1] Methazolamide is a cousin of the widely used and successful AMS/HAPE/HACE preventing drug acetazolamide (Diamox).[1,7,8] The advantages of methazolamide are that lower doses with fewer side effects seem to achieve equivalent protective effects in animal and human studies. **Why consider these two drugs used in combination?** Our colleague Dr. Dave Irwin recently showed that the combination of nifedipine + methazolamide was the most effective of all compounds besides dexamethasone in preventing HAPE and HACE in a rat model of these diseases. This combination of two FDA approved drugs has never been tested in humans. Based on the animals studies by Dr. Irwin and the effectiveness of each drug alone in humans **we think the combination of nifedipine + methazolamide has considerable potential to be effective in protecting humans from all the high-altitude illnesses.**

What is metformin, and why might it work to aid acclimatization and prevent high-altitude illnesses? Working together with Yang Xia at UT Houston we had the opportunity to study oxygen transport modulation during human acclimatization to high altitude. Since acclimatized humans are nearly completely protected from high-altitude illness and have better physical and cognitive function we reasoned that any factor responsible for enabling acclimatization would potentially play a role in invoking acclimatization–like responses in altitude-naïve subjects, such as warfighters being rapidly deployed to high altitude.

To function effectively in O_2 uptake and release, erythrocytes rely on sophisticated regulation of hemoglobin (Hb)-O_2 affinity by allosteric modulators. One of the best-known allosteric modulators is 2,3-bisphophosphoglycerate (2,3-BPG). Earlier studies demonstrated that erythrocyte 2,3-BPG levels are elevated at a high altitude and in sickle cell disease.[9] However, factors responsible for 2,3-BPG induction at high altitude are unknown. To address this question, we tested 21 individuals placed at high altitude for different periods of time. We found that: 1)

Plasma adenosine and erythrocyte 2,3-BPG levels were significantly elevated in normal individuals following 24 hours at high altitude compared to sea level. 2) Adenosine and 2,3-BPG levels were further enhanced after 16 days at high altitude. 3) Elevated circulating adenosine levels significantly correlated with increased erythrocyte 2,3-DPG levels in normal individuals at high altitude. Next, we found *in vitro* evidence that adenosine signaling via A_{2B} adenosine receptors (ADORA2B) induced 2,3-BPG production in a protein kinase A (PKA) and AMP activated protein kinase (AMPK)-dependent manner in cultured human erythrocytes. These studies show that adenosine signaling through ADORA2B regulates erythrocyte 2,3-BPG induction as a function of altitude and identifies new targets to enhance O_2 release under hypoxia conditions.

Metformin is an FDA approved drug to treat diabetic patients that decreases hyperglycemia primarily by suppressing glucose production by the liver.[10] Although the molecular basis of metformin is not fully understood, it is well known to activate AMPK and PKA. Based on our initial observation that PKA and AMPK are essential to regulate 2,3-BPG induction and promote O_2 release from erythrocytes, we propose to test the intriguing possibility that metformin is a safe drug to induce O_2 release and thereby mimic acclimatization and and prevent high-altitude sickness.

Overall Study Approach

We will test 60 young men and women resembling young, fit military recruits for the effectiveness of three compounds versus placebo (15 in each group). Each compound has been selected for strong scientific rationale of potential effectiveness and immediacy of availability for use by the SOF warfighter operating at high altitudes. Human studies are difficult, expensive and time consuming. Our team has developed considerable expertise in this area and can deliver a thorough evaluation of these compounds with the goal of rapidly improving SOCCOM options for dealing with high altitude medical problems.

3. Bibliography. (limit: list of 10 documents)

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4. Goals and/or Objectives. (limit: 20 lines of text)

Goal 1: Evaluate effectiveness for preventing AMS, HAPE and HACE of quercetin, nifedipine+methazolamide and metformin compared to placebo during a series of tests of performance at high altitude.

Objective 1. Evaluate effectiveness of the neutraceutical quercetin to prevent high altitude illness, and improve physical and cognitive performance upon rapid ascent and over three days at altitudes between 10,000 and 13,000 feet.

Objective 2. Evaluate a comnbination of nifedipine+methazoalmide, FDA approved drugs that have been shown alone to help with HAPE (nifedipine) and AMS/HACE (methazolamide), to prevent high-altitude illness, and improve physical and cognitive performance upon rapid ascent and over three days at altitudes between 10,000 and 13,000 feet.

5. Work. (limit: one page of text)

Work to be accomplished will include:

1. Obtain University of Colorado and Oregon IRB approval for study.
2. Obtain DOD HRPO approval for study.
3. Recruit 60 volunteers from University of Oregon community at sea level. All subjects will pass rigorous physical screening, including mandatory passage of Army PFT at age-specific levels or SOCCOM directed Special Forces fitness assessment.
4. Carefully screen Oregon volunteers for inclusion/exclusion criteria.
5. Select Oregon and Colorado trails with similar elevation gain over three miles.
6. Schedule Oregon volunteers for trip to Colorado weekends in Colorado.
7. Establish Colorado basecamp in Breckenridge, Colorado.
8. Conduct test weekend with Oregon staff in Colorado to review and revise all pertinent procedures.
9. Begin serial weekend testing for 5 to 10 weekends over 12 weeks, with 10-20 subjects per group.
10. Conduct daily data analysis and interpretation of AMS, physical performance and cognitive function data.
11. Finish series of weekend tests.
12. Complete data entry, prepare for data analysis.

13. Break drug code and discover which of the proposed compounds had a substantial effect on AMS, HAPE and HACE prevention, physical performance or cognitive performance in real world field conditions at high altitude compared to placebo.

This ambitious plan is fully realizable because of our recent experience conducting three DoD-TATRC-funded field studies. In those study we tested whether a test of gene expression at sea level could predict who would later develop AMS. In the first of two field trials for that study we could predict 9/10 young, healthy civilians who would either get sick with AMS or stay healthy. The validation of that study was just completed and analyses are underway. If successful, a test will be developed for broad military and civilian use to predict at sea level susceptibility of AMS. The third study was a massive effort to study 21 subjects at sea level and after several weeks at high altitude at 17,200 feet in Bolivia. The results from that study are the first description of the molecular mechanisms responsible for the human body's response to prolonged hypoxia and have led us to propose metformin as a novel candidate drug for improving human performance at high altitude. We conducted both of those studies at the same time, managed to get IRB and HRPO clearance for both in a reasonably timely manner, and have met all milestones for the proposed studies. This success gives a great confidence that we can complete the proposed studies as outlined here.

6. Deliverables. (limit: 20 lines of text)
We will deliver interim reports every quarter on study progress. At the completion of 18 months, we will deliver preliminary results form the field trials. At 24 months we will present a final report including analysis of all results from all trials, a presentation suitable for wide distribution of the final results, and executive recommendations for implementations of the findings for immediate SOCCOM application.

7. Government Furnished Property. (limit: 20 lines of text)
Not applicable.

8. Place of Performance. (limit: 5 lines of text)
University of Colorado Anschutz Medical Campus, Aurora, Colorado; field basecamp in Breckenridge, Colorado; and University of Oregon, Department of Human Physiology, Eugene, Oregon.

9. Period of Performance. (limit: 5 lines of text)
24 months. Six months for project startup and IRB + HRPO approvals, 12 months for field data collection, 6 months for data analysis and report writing. This schedule allows time for schedule slippage if DoD HRPO is slow in processing IRB approval, and allows ample time for our experienced team to recruit subjects, collect data in the field and analyze the data for efficacy in preventing high altitude illness and improving physical and cognitive performance.

10. Human Use and Animal Use. (limit: 20 lines of text)
We will conduct human experiments, similar in nature to our recent DoD-funded and HRPO approved field studies in South America and in Breckenridge, Colorado. We have a template in place of HRPO approved procedures and protocols for every aspect of the proposed studies except the specific risks of the proposed compounds. Furthermore, if we are selected to present a

full proposal we will simultaneously prepare an IRB submission for presentation to our local IRB on the day we receive notification of funding. Since this will be nearly identical to previously approved studies we expect to have local IRB approval within three months of notification of funding, and HRPO approval hopefully within three to six months of submission to HRPO. Since we will start the IRB process on 'notification of funding', which is likely several months before 'start of funding', we will gain an extra few critical months on the funded timeline to allow for idiosyncrasies in HRPO processing.

11. Principal Investigator (PI) and Support Personnel. (limit: 20 lines of text to list personnel and their contact information)

Robert Roach, PhD. Principal Investigator, Director, Altitude Research Center, University of Colorado Anschutz Medical Campus, rroach@hypoxia.net

Andrew Subudhi, Ph.D. Co-Principal Investigator. Jointly responsible with PI for overall conduct of the study. Associate Professor, University of Colorado, Colorado Springs, asubudhi@uccs.edu

Andrew Lovering, Ph.D., Co-Investigator, responsible for subject recruitment, screening and management. Associate Professor, Department of Human Performance, University of Oregon, lovering@uoregon.edu

Yang Xia, MD, PhD, Co-Investigator. Responsible for analysis and interpretation of metformin results in comparison with findings in animal and other human studies of adenosine and acclimatization, yang.xia@uth.tmc.edu

David Irwin, PhD, Consultant on translation of DARPA-funded drug discovery for protection from high altitude illness from rodents to humans, Department of Medicine, University of Colorado Anschutz Medical Campus, David.Irwin@ucdenver.edu

Mr. Rod Alne, US Air Force CMSgt Pararescue, Ret, a former Air Force pararescue specialist with extensive SOF and high altitude experience will serve as our special forces applications advisor, rod.alne@thepeakinc.com

12. Budget. (limit: 20 lines of text)

Direct Cost University of Colorado Denver: These costs will include all field logistics, room rental, food and transport costs both local in Colorado and from Oregon to Colorado. Colorado staff will be primarily responsible for the Colorado IRB and DOD HRPO IRB document preparation. Colorado staff will be primarily responsible for all DOD reporting. Colorado staff will be primarily responsible for all data entry and analysis, and final interpretation and recommendations to the DOD.

Subcontract University of Oregon. This will include staff to recruit, screen and pre-test 100 subjects to get a clean pool of 60 research subjects who can pass all inclusion/exclusion criteria, including the modified Army PFT. The Oregon budget will also include support for the Oregon IRB submission. Subject payments will also be made by University of Oregon.

	Year 1	Year 2	Grand Total
Direct Costs UC Denver	$ 195,000	$ 170,000	$ 365,000
Subcontract U of O	$ 90,000	$ 35,000	$ 125,000
Total Direct Costs	$ 285,000	$ 200,000	$ 485,000
Total Indirect Costs	$ 121,000	$ 93,500	$ 214,500
Grand total	$ 406,000	$ 293,500	$ 699,500

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Robert C. Roach, Ph.D.	Associate Professor
eRA COMMONS USER NAME ROACH.R	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The Evergreen State College, Olympia, WA	B.S.	1979	Biochemistry
Cornell University, Ithaca, NY	M.S.	1985	Nutritional Science
University of New Mexico, Albuquerque, NM	Ph.D.	1994	Exercise Physiology

A. Personal Statement

My research focuses on the broad area of human responses to hypoxia. Current research is focused on three major areas: cerebrovascular hemodynamics in hypoxia and exercise; transcriptomic prediction of human responses to hypoxia; and the integration of systems biology with physiology to understand the molecular and cellular mechanisms of oxygen sensing in humans. We have recently shown that hypoxia impairs cerebral autoregulation; research is underway to begin to understand the importance of this finding and its possible mechanisms. We found that a gene expression signature from a blood sample collected in Denver predicted >95% of those who later developed acute mountain sickness. A recent validation study at lower altitudes in a more diverse population confirmed these findings. Studies are underway to examine the physiological links of these transcriptomic markers and pathways that lead to susceptibility to altitude illness. And finally, we are undertaking studies to link a comprehensive 'omics' pathway of oxygen sensing (transcriptomics, epigenetics, proteomics, metabolomics) to physiological responses that serve to improve oxygen transport during acute and chronic hypoxia. In my position as Research Director and now Director of the Altitude Research Center I have mentored 17 researchers, ranging from research fellows in Emergency Medicine, Neurology and Pulmonary Medicine to medical students, postdoctoral fellows and undergraduate students. I am active on the Department of Emergency Medicine research council, and mentor for an additional six junior faculty on routine research-related topics.

B. Positions and Honors

Positions and Employment

2010-present	Director, Altitude Research Center, University of Colorado Denver, Denver, CO
2003-2010	Associate Director and Chief, Research Division, Altitude Research Center UCDHSC, Denver, CO
2001-2003	Scientist, New Mexico Resonance, Albuquerque, NM
1999-present	Co-Chairman, International Hypoxia Symposia (www.hypoxia.net)
1999-2002	Clinical Assistant Professor, Department Surgery, Div Emergency Medicine, UCDHSC,O
1999-2003	Clinical Assistant Professor, Department Medicine, University of New Mexico, Albuquerque, NM
1999-2000	Research Assistant Professor, Department Life Sciences, New Mexico Highlands University, Las Vegas, NM
1998-1999	Visiting Professor, Department of Life Sciences, New Mexico Highlands University, Las Vegas, NM
1996-1998	Alfred Benzon Research Fellow, Copenhagen Muscle Research Center, Copenhagen, Denmark.
1994-1996	Associate Scientist, Cardiopulmonary Physiology, Institute Basic Applied Medical Research, The Lovelace Institutes, Albuquerque, NM
1993-2005	Consultant, Life Support Systems, Odyssey Around the World Balloon Flight, Albuquerque, NM
1990-1994	Research Physiologist, Oxygen Transport Program, The Lovelace Institutes, Albuquerque, NM
1989-2005	Associate Scientist, Siberian-Alaskan Medical Research Exchange, Section Cold Altitude Physiology, University of Alaska, Anchorage, AK

1982-1990 Associate Director, Denali Medical Research Project, University of Alaska, Anchorage, AK

Professional Memberships

American Physiological Society; American College of Sports Medicine; American Association for the Advancement of Science; American Alpine Club; International Society for Mountain Medicine

Review and Referee Work

Appointed, Editorial Board, Journal of Applied Physiology, 2006 to present
Appointed, Editorial Board, Medicine Science Sports and Exercise, 2005 to 2011
Appointed, Section Editor, Hypoxia and High Altitude, Extreme Medicine and Physiology, BMC Journals, 2011-present.
Invited Reviewer, DOD Brain Injury Study Section, American Institute of Biological Science, 2009-2010.

Honors and Awards

Elected Fellow, American College of Sports Medicine (FACSM), fall 2004.
Appointed, American Physiological Society Porter Scholarship Selection Committee, 2005-2008
Appointed, American College of Sports Medicine, Constitution, Bylaws and Operating Codes Committee, 2006-2009
Appointed, American College of Sports Medicine, Promotions and Fellowship Committee, 2011 to present

C. Selected Peer-Reviewed Publications (selected from 86 peer-reviewed publications)
Most relevant to the current application

Roach EB, Bleiberg J, Lathan CE, Wolpert L, Tsao JW, **Roach R.C.**, AltitudeOmics: Decreased reaction time after high altitude cognitive testing is a sensitive metric of hypoxic impairment. Neuroreport. 2014 Apr 9

Subudhi, A., J. Bucher, N. Bourdillon, C. Davis, J. Elliott, M. Eutermoster, O. Evero, J.L. Fan, S. Jameson-Van Houten, C.G. Julian, J. Kark, S. Kark, B. Kayser, J.P. Kern, S.E. Kim, C. Lathan, S.S. Laurie, A.T. Lovering, R. Paterson, D. Polaner, B.J. Ryan, J. Spira, N.B. Wachsmuth, and **Roach R.C.**, AltitudeOmics: The Integrative Physiology of the Onset and Retention of Acclimatization to Hypoxia in Humans. PLoS One, 2014; 9(3).

Roach R.C., Wagner PD, Hackett PH Translation in progress: hypoxia. J Appl Physiol (1985). 2014 Apr 1;116(7)

Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness Journal of Applied Physiology, 2014; 117(7).

Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Effect of ascent and acclimatization to 5,260 m on regional cerebral oxygen delivery Experimental Physiology, 2014.

Julian, C.G., A.W. Subudhi, R.C. Hill, M.J. Wilson, A.C. Dimmen, K.C. Hansen, and **R.C. Roach**, Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. Journal of Applied Physiology, 2013; 116(7).

Goodall, S., M. Amann, R. Twomey, A.W. Subudhi, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude Acta Physiologica, 2014; 210(4).

Fan, J.L., A. Subudhi, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high altitude acclimatisation and re-exposure Journal of Applied Physiology, 2014; 116(7).

Davis, C., E. Reno, B. Vestal, E. Maa, and **R.C. Roach**, Risk Factors for Headache While Hiking Above 4300 Meters. High Altitude Medicine and Biology (in review), 2013.

Wilson MJ, Julian CG, **Roach R.C**. Genomic analysis of high altitude adaptation: innovations and implications. Curr Sports Med Rep 2011;10:59-61.

Subudhi AW, Dimmen AC, Julian CG, Wilson MJ, Panerai RB, **Roach R.C**. Effects of acetazolamide and dexamethasone on cerebral hemodynamics in hypoxia. J Appl Physiol 2011;110:1219-25.

Roach R.C., Kayser B, Hackett P. Pro: Headache should be a required symptom for the diagnosis of acute mountain sickness. High Alt Med Biol 2011;12:21-2.

Julian CG, Subudhi AW, Wilson MJ, Dimmen AC, Pecha T, **Roach R.C**. Acute Mountain Sickness,

Inflammation and Permeability: New Insights from a Blood Biomarker Study. J Appl Physiol 2011.
Subudhi AW, Panerai RB, **Roach R.C**. Effects of Hypobaric Hypoxia on Cerebral Autoregulation. Stroke 2010.
Subudhi AW, Panerai RB, **Roach R.C**. Acute hypoxia impairs dynamic cerebral autoregulation: results from two independent techniques. J Appl Physiol 2009;107:1165-71.
Subudhi AW, Miramon BR, Granger ME, **Roach R.C**. Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. J Appl Physiol 2009;106:1153-8.
Loeppky JA, Icenogle MV, Charlton GA, Conn CA, Maes D, Riboni K, Gates L, Melo MFV, **Roach R.C**. Hypoxemia and acute mountain sickness: which comes first? High Alt Med Biol 2009;9:271-9.

Additional recent publications of importance to the field

Ezzati M, Horwitz MEM, Thomas DSK, Friedman AB, **Roach RC**, Clark T, Murray CJL, Honigman B. Altitude, life expectancy and mortality from ischaemic heart disease, stroke, COPD and cancers: national population-based analysis of US counties. Journal Epidemiology Community Health 2011.
Browne VA, Toledo-Jaldin L, Davila RD, Lopez LP, Yamashiro H, Cioffi-Ragan D, Julian CG, Wilson MJ, Bigham AW, Shriver MD, Honigman B, Vargas E, **Roach RC**, Moore LG. High-end arteriolar resistance limits uterine artery blood flow and restricts fetal growth in preeclampsia and gestational hypertension at high altitude. Am J Physiol Regul Integr Comp Physiol 2011;300:R1221-9.
Asgari S, Subudhi AW, **Roach RC**, Liebeskind DS, Bergsneider M, Hu X. An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. J Neurosci Methods 2011;197:171-9.
Roach RC. Hypoxia and the Circulation. New York: Springer; 2007.
Hackett PH, **Roach RC**. High Altitude Medicine. In: Auerbach PS, ed. Wilderness Medicine. Vol 6th. Philadelphia: Mosby Elsevier; 2011:2-36.
Subudhi AW, Panerai RB, **Roach RC**. Effects of Hypobaric Hypoxia on Cerebral Autoregulation. Stroke 2010.
Imray C, Wright A, Subudhi A, **Roach RC**. Acute mountain sickness: pathophysiology, prevention, and treatment. Progress in Cardiovascular Diseases 2010;52:467-84.
Tissot van Patot MC, Serkova NJ, Haschke M, Kominsky DJ, **Roach RC**, Christians U, Henthorn TK, Honigman B. Enhanced leukocyte HIF-1alpha and HIF-1 DNA binding in humans after rapid ascent to 4300 m. Free Radic Biol Med 2009;46:1551-7.
Subudhi AW, Panerai RB, **Roach RC**. Acute hypoxia impairs dynamic cerebral autoregulation: results from two independent techniques. J Appl Physiol 2009;107:1165-71.
Subudhi AW, Miramon BR, Granger ME, **Roach RC**. Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. J Appl Physiol 2009;106:1153-8.
Hu X, Subudhi AW, Xu P, Asgari S, **Roach RC**, Bergsneider M. Inferring cerebrovascular changes from latencies of systemic and intracranial pulses: a model-based latency subtraction algorithm. J Cereb Blood Flow Metab 2009;29:688-97.
Subudhi AW, Lorenz MC, Fulco CS, **Roach RC**. Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. Am J Physiol Heart Circ Physiol 2008;294:H164-71.
Firth PG, Zheng H, Windsor JS, Sutherland AI, Imray CH, Moore GWK, Semple JL, **Roach RC**, Salisbury RA. Mortality on Mount Everest, 1921-2006: descriptive study. BMJ 2008;337:a2654.
Subudhi AW, Dimmen AC, **Roach RC**. Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. J Appl Physiol 2007;103:177-83.
Roach RC. Hypoxia and Exercise. New York: Springer; 2005.
Loeppky JA, Icenogle MV, Maes D, Riboni K, Hinghofer-Szalkay H, **Roach RC**. Early fluid retention and severe acute mountain sickness. J Appl Physiol 2005;98:591-7.
Roach RC. Hypoxia Through the Lifecycle. New York: Kluwer Academic Plenum Publishers; 2003.
Møller K, Paulson OB, Hornbein TF, Colier WNJM, Paulson AS, **Roach RC**, Holm S, Knudsen GM. Unchanged cerebral blood flow and oxidative metabolism after acclimatization to high altitude. J Cereb Blood Flow Metab 2002;22:118-26.
Roach RC, Stepanek J, Hackett P. Acute Mountain Sickness And Cerebral Edema. (Rock P, Pandolf K, eds.). Washington, DC: US Army; 2002.
Bestle MH, Olsen NV, Poulsen TD, **Roach RC**, Fogh-Andersen N, Bie P. Prolonged hypobaric hypoxemia attenuates vasopressin secretion and renal response to osmostimulation in men. J Appl Physiol 2002;92:1911-22.

Hackett PH, **Roach RC**. High-altitude illness. N Engl J Med 2001;345:107-14.
Roach RC, Maes D, Sandoval D, Robergs RA, Icenogle M, Hinghofer-Szalkay H, Lium D, Loeppky JA. Exercise exacerbates acute mountain sickness at simulated high altitude. J Appl Physiol 2000;88:581-5.
Roach RC. Hypoxia: Into the Next Millenium. (Wagner P, Hackett P, eds.). NY: Plenum/Kluwer Academic; 1999.
Roach RC, Koskolou MD, Calbet JAL, Saltin B. Arterial O2 content and tension in regulation of cardiac output and leg blood flow during exercise in humans. Am J Physiol 1999;276:H438-45.
Roach RC, Greene ER, Schoene RB, Hackett PH. Arterial oxygen saturation for prediction of acute mountain sickness. Aviation, space, and environmental medicine 1998;69:1182-5.
Koskolou MD, **Roach RC**, Calbet JAL, Rådegran G, Saltin B. Cardiovascular responses to dynamic exercise with acute anemia in humans. Am J Physiol 1997;273:H1787-93.
Koskolou MD, Calbet JAL, Rådegran G, **Roach RC**. Hypoxia and the cardiovascular response to dynamic knee-extensor exercise. Am J Physiol 1997;272:H2655-63.
Roach RC, Loeppky JA, Icenogle MV. Acute mountain sickness: increased severity during simulated altitude compared with normobaric hypoxia. J Appl Physiol 1996;81:1908-10.
Loeppky JA, Scotto P, **Roach RC**. Acute ventilatory response to simulated altitude, normobaric hypoxia, and hypobaria. Aviation, space, and environmental medicine 1996;67:1019-22.
Honigman B, Theis MK, Koziol-McLain J, **Roach RC**, Yip R, Houston C, Moore LG, Pearce P. Acute mountain sickness in a general tourist population at moderate altitudes. Ann Intern Med 1993;118:587-92.
Loeppky JA, Scotto P, Riedel CE, **Roach RC**, Chick TW. Effects of acid-base status on acute hypoxic pulmonary vasoconstriction and gas exchange. J Appl Physiol 1992;72:1787-97.
Grissom CK, **Roach RC**, Sarnquist FH, Hackett PH. Acetazolamide in the treatment of acute mountain sickness: clinical efficacy and effect on gas exchange. Ann Intern Med 1992;116:461-5.
Schoene RB, Swenson ER, Pizzo CJ, Hackett PH, **Roach RC**, Mills WJ, Henderson WR, Martin TR. The lung at high altitude: bronchoalveolar lavage in acute mountain sickness and pulmonary edema. J Appl Physiol 1988;64:2605-13.
Hackett PH, **Roach RC**, Schoene RB, Harrison GL, Mills WJ. Abnormal control of ventilation in high-altitude pulmonary edema. J Appl Physiol 1988;64:1268-72.
Schoene RB, Hackett PH, Henderson WR, Sage EH, Chow M, **Roach RC**, Mills WJ, Martin TR. High-altitude pulmonary edema. Characteristics of lung lavage fluid. JAMA 1986;256:63-9.
Schoene RB, **Roach RC**, Hackett PH, Harrison G, Mills WJ. High altitude pulmonary edema and exercise at 4,400 meters on Mount McKinley. Effect of expiratory positive airway pressure. Chest 1985;87:330-3.
Roach RC, Larson EB, Hornbein TF, Houston CS, Bartlett S, Hardesty J, Johnson D, Perkins M. Acute mountain sickness, antacids, and ventilation during rapid, active ascent of Mount Rainier. Aviation, space, and environmental medicine 1983;54:397-401.
Larson EB, **Roach RC**, Schoene RB, Hornbein TF. Acute mountain sickness and acetazolamide. Clinical efficacy and effect on ventilation. JAMA 1982;248:328-32.

D. Ongoing Research Support

DMDRP W81XWH-11-2-0034 (Roach, PI) 12/20/2010-6/30/2014
Prediction of acute mountain sickness using a blood-based test
This project aims at developing a rapid, cost-effective, pre-ascent screening test to predict individual risk of acute mountain sickness (AMS) for military use.

DMDRP W81XWH-11-2-0040 (Roach, PI) 01/01/2011-6/30/2014
AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude
This project aims at advancing high-altitude medical research by discovering the basic molecular mechanisms of acclimatization that protect soldiers from high altitude illness.

DARPA (**PI: Irwin, D, Co-PI: Roach**) (01/01/2012-12/31/2014)
Rapid Acclimatization to Hypoxia at Altitude.
The advancement of high-altitude medical research by discovering novel preventive measures for acute mountain sickness.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Andrew W. Subudhi	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) asubudhi	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
The Colorado College	B.A.	1992	Mathematics
Colorado State University	M.S.	1996	Exercise Science
University of Utah	Ph.D.	2000	Exercise Physiology
University of Colorado Health Science Center	Post Doc	2003-5	Altitude Physiology

A. Personal Statement

My doctoral training in exercise physiology has given me a solid foundation for assessing cardiovascular, respiratory, and neuromuscular physiology in human subjects. During my PhD training, and for 5 years afterwards, my primary responsibility was monitoring training adaptations of athletes who were living and training at altitude in preparation for the 1998, 2002 and 2006 Olympic Winter Games. Based on my work in this area, I was invited to participate with research teams from Stanford University and the United States Army to study the physiological effects of acute altitude exposure and acclimatization. These experiences fueled my motivation to pursue a post-doctoral training under the direction of Dr. Robert Roach at the University of Colorado Altitude Research Center (ARC). While at ARC, I learned several techniques for assessing cerebrovascular physiology. Since securing a tenure-track position within the University of Colorado, I have developed a line of research investigating the influence of cerebral blood flow and oxygenation on health and performance at altitude. Given my breadth of knowledge and skill in assessing integrative physiological responses to hypoxia, I am particularly well prepared and suited for my role in this project.

B. Positions and Honors

<u>Positions</u>

1997 - 2005 Research Scientist, The Orthopedic Specialty Hospital (TOSH), Intermountain Health Care, Salt Lake City, UT.
2000 - 2008 Adjunct Assistant Professor, University of Utah, Division of Foods & Nutrition, Salt Lake City, UT.
2001 - 2005 Adjunct Assistant Professor, University of Utah, Dept. of Exercise & Sport Science, Salt Lake City, UT.
2005 - 2011 Assistant Professor, University of Colorado at Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2005 - 2011 Assistant Professor, University of Colorado at Denver, Dept. of Surgery, Denver, CO.
2011 - Present Associate Professor, University of Colorado Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2011 - Present Associate Professor, University of Colorado Denver/Anschutz Medical Campus, Dept. of Emergency Medicine, Denver, CO.

<u>Professional Memberships</u>

1995 – Present Member of the American College of Sports Medicine
1996 – Present Certified Strength and Conditioning Specialist (C.S.C.S.)
2000 – Present Member of the American Physiological Society

C. Selected Peer-Reviewed Publications (selected from 43 peer-reviewed publications)

<u>Most relevant to the current application</u>

- Subudhi, A., J. Bucher, N. Bourdillon, C. Davis, J. Elliott, M. Eutermoster, O. Evero, J.L. Fan, S. Jameson-Van Houten, C.G. Julian, J. Kark, S. Kark, B. Kayser, J.P. Kern, S.E. Kim, C. Lathan, S.S. Laurie, A.T. Lovering, R. Paterson, D. Polaner, B.J. Ryan, J. Spira, N.B. Wachsmuth, and Roach R.C.(2014) AltitudeOmics: The Integrative Physiology of the Onset and Retention of Acclimatization to Hypoxia in Humans. PLoS One; 9(3).

- Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and R.C. Roach (2014) AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness Journal of Applied Physiology; 117(7).

- Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, A.T. Lovering, and R.C. Roach (2014) AltitudeOmics: Effect of ascent and acclimatization to 5,260 m on regional cerebral oxygen delivery Experimental Physiology.

- Julian, C.G., A.W. Subudhi, R.C. Hill, M.J. Wilson, A.C. Dimmen, K.C. Hansen, and R.C. Roach (2014) Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. Journal of Applied Physiology; 116(7).

- Goodall, S., M. Amann, R. Twomey, A.W. Subudhi, A.T. Lovering, and R.C. Roach, AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude Acta Physiologica, 2014; 210(4).

- Fan, J.L., A. Subudhi, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and R.C. Roach (2014) AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high altitude acclimatisation and re-exposure Journal of Applied Physiology, 116(7).

- Subudhi, A.W., Dimmen, A.C., & Roach, R.C. (2007) Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. Journal of Applied Physiology, 103(1), 177-183.

- Amann, M., Romer, L.M., Subudhi, A.W., Pegelow, D.F., & Dempsey, J.A. (2007). Severity of arterial hypoxemia affects the relative contributions of peripheral vs. central fatigue to exercise performance. Journal of Physiology, 581(1), 389-403.

- Subudhi, A.W., Lorenz, M.C., Fulco, C.S., & Roach, R.C. (2008). Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. American Journal of Physiology - Heart and Circulatory Physiology, 294(1), H164-H171.

- Subudhi, A.W., Miramon, B.R., Granger, M.E., & Roach, R.C. (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. Journal of Applied Physiology, 106(4), 1153-1158.

- Subudhi, A.W., Panerai, R.B, & Roach, R.C. (2009). Acute hypoxia impairs dynamic cerebral autoregulation: Results from two independent techniques. Journal of Applied Physiology, 107(4), 1165-1171.

- Hu, X., Subudhi, A., Xu, P., Asgari, S., Roach, R., & Bergsneider, B. (2009). Inferring cerebrovascular changes from latencies of systemic and intracranial pulses: a model based latency subtraction algorithm. Journal of Cerebral Blood Flow and Metabolism, 29(4), 688-697.

- Subudhi, A.W., Panerai, R.B, & Roach, R.C. (2010). Effects of hypobaric hypoxia on cerebral autoregulation. Stroke**,** published ahead of print 10.1161/STROKEAHA.109.574749.

- Subudhi, A.W., Dimmen, A.C., Julian, C.G., Wilson, M.J., Panerai, R.B., & Roach, R.C. (2011). Effects of acetazolamide and dexamethasone on cerebral hemodynamics in hypoxia. Journal of Applied Physiology, 110(5): 1219-1225.

- Subudhi, A.W., Olin J.T., Dimmen, A.C., Polaner, D.M., Kayser, B., & Roach, R.C. (2011). Does cerebral oxygen delivery limit incremental exercise performance? Journal of Applied Physiology. 111(6): 1727-1734.

- Asgari, S., Subudhi, A.W., Xu, P., Roach, R.C., Liebeskind, D.S., Bergsneider, B., & Hu, X. (2011). An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. Journal of Neuroscience Methods, 197(1), 171-179.

- Sato, K., Sadamoto, T., Hirasawa, A., Oue, A., Subudhi, A.W., Miyazawa, T., & Ogoh, S. (2012). Differential blood flow responses to CO_2 in human internal and external carotid and vertebral arteries. Journal of Physiology, 590(Pt14): 3277-3290.

- Asgari, S., Gonzalez, N., Subudhi, A.W., Hamilton, R., Vespa, P., Bergsneider, M., Roach, R.C., & Hu, X. (2012). Continuous detection of cerebral vasodilatation and vasoconstriction using intracranial pulse morphological template matching.PLoS One, 7(11):e50795.

- Miyazawa, T., Horiuchi, M., Ichikawa, D., Subudhi, A.W., Sugawara, J., & Ogoh, S. (2012). Face cooling with water mist increases cerebral blood flow during exercise: effect of changes in facial skin blood flow. Frontiers in Physiology, 3:308.

- Ogoh, S., Sato, K., Nakahara, H., Okazaki, K., Subudhi A., & Miyamoto, T. (2013). Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. Experimental Physiology. 98.3: 692-698.

- Amann, M., Goodall, S., Twomey, R., Subudhi, A.W., Lovering, A.T., & Roach, R.C. (2013). AltitudeOmics: On the consequences of high altitude acclimatization for the development of fatigue during locomotor exercise in humans. Journal of Applied Physiology, 115(5): 634-42.

- Subudhi, A.W., Fan, J.L., Evero, O., Bourdillon, N., Kayser, B., Julian, C.G., Lovering, A.T., & Roach, R.C. (in press). AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery**.** Experimental Physiology

D. Research Support

<u>Current Support</u>

2009 – Present	Medical Education and Research Institute of Colorado (MERIC). Interdisciplinary laboratory investigations in biological sciences. Role: Co-Principal Investigator (w/ Jacqueline Berning and Jeffery Broker).
2010 - 2014	Department of Defense - Applied Research and Advanced Technology Development Award. Prediction of Acute Mountain Sickness Using a Blood-Based Test. Role: Co-Investigator (PI: Robert C. Roach).
2011 - 2014	Department of Defense - Defense Medical Research and Development Program Basic Research Award. AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude. Co-Investigator (PI: Robert C. Roach).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Andrew T. Lovering, PhD	POSITION TITLE Associate Professor of Human Physiology
eRA COMMONS USER NAME (credential, e.g., agency login) LOVERING	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Texas Tech University (TTU), Lubbock, TX	BS	05/95	Biology
TTU School of Medicine (TTUSOM), Lubbock, TX	PhD	05/03	Neurophysiology
University of Wisconsin (UW) School of Medicine and Public Health, Madison, WI	Postdoctoral	06/07	Pulmonary Physiology

A. Personal Statement

The goal of the proposed research is to find the best new approach to improving SOF warfighter performance on rapid exposure to high altitude.

The Lovering Lab is well suited to provide critical expertise towards the aims of this project as I have an extensive background in cardiopulmonary physiology, with specific interest in physiological responses to high altitude and hypoxia. Our lab location is also well suited for the recruitment and screening of altitude naïve subjects. In fact, we have previously worked with Dr. Roach to select and screen altitude naïve subjects for the AltitudeOmics 2012 research expedition to Bolivia. Research conducted in my lab related to hypoxia, high altitude and lung disease has been well funded by the DOD, the AHA, and the ALA. Importantly, we are completing work on a recent grant from the Defense Medical Research & Development Program to study the role of intrapulmonary arteriovenous anastomoses and patent foramen ovale with relation to pulmonary gas exchange efficiency and acute mountain sickness. I have also participated in field research expeditions in Nepal and Tibet to study high altitude physiology through collaborations with other leading scientists in the field. These collaborations have resulted in several peer-reviewed publications and ongoing projects. My previous experiences with team-based research projects have taught me the importance of clear communication between project members, constructing achievable research plans, goals, and budgets.

B. Positions and Honors

Positions and Employment

1993 – 1995 Undergraduate Fellow, TTUSOM, Dept of Pharmacology, Lubbock, TX
1995 – 1996 Research Technician II, TTUSOM, Dept of Pharmacology, Lubbock, TX
1996 – 1998 Research Technician III, TTU, Biology Dept, Lubbock, TX
1998 – 2003 Graduate Research Fellow, TTUSOM, Dept of Physiology, Lubbock, TX
2003 – 2007 Postdoctoral Fellow, UW School of Medicine & Public Health, Madison, WI
2007 – 2012 Assistant Professor, University of Oregon, Human Physiology, Eugene, OR
2012 – Present Associate Professor, University of Oregon, Human Physiology, Eugene, OR

Other Experience and Professional Memberships

1999 – Present American Physiological Society (APS) Member
2005 APS Minority Travel Fellowship Mentor for Carmen Troncoso
2012 NIGMS Minority Summer Fellow Mentor for Juan Wilkins

Honors

1993 – 1995	ASPET Summer Fellowship for Undergraduate Research
1998 – 2003	U.S. Department of Education GAANN Fellowship
2000	Travel Award, IV World Congress; Mount Med & High Altitude Physiol–Chile
2001	Travel Award, World Federation of Sleep Res. Societies III Conf–Uruguay
2002 – 2003	Achievement Rewards for College Scientists (ARCS) Foundation Scholarship
2003	Sleep Research Society Trainee Research Merit Award – Chicago
2003	The Outstanding Graduate Student, TTUSOM
2003 – 2006	NIH Postdoctoral Fellowship in Respiratory Neurobiology
2005 – 2007	NIH Clinical Loan Repayment Program
2010	Sacred Heart Foundation PeaceHealth Clinical Research Recognition Award
2010	APS Giles F. Filley Memorial Awards for Excellence in Resp Physiol & Med
2012	University of Oregon Faculty Excellence Award

C. Selected Peer-reviewed Publications (Selected from 39 peer-reviewed publications)

Most Relevant to the Current Application

1. Subudhi, A.W., J-L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, **A.T. Lovering**, R. Panerai R.C. Roach. AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness" *J Appl Physiol* 116: 724-729, 2014. PMID: 24371013
2. Fan, J-L, A.W. Subudhi, O. Evero, N. Bourdillon, B. Kayser, **A.T. Lovering**, R.C. Roach. AltitudeOmics: Enhanced cerebrovascular function with high altitude acclimatisation and re-exposure *J Appl Physiol* 116: 911-918, 2014. PMID: 24356520
3. Subudhi, A.W., J-L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, **A.T. Lovering**, R.C. Roach. AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. *Exp Physiol 99 (5) 772-781;2014.* PMID: 24243839
4. Subudhi, A.W., J. Bucher, N.Bourdillon, C.Davis, J.Elliott, M.Eutermoster, O.Evero, J-L. Fan, S.Jameson-Van Houten, C.G. Julian, J.Kark, S.Kark, B.Kayser, J.P. Kern, S. Kim, C. Lathan, S.S. Laurie, **A.T. Lovering**, R. Paterson, D. Polaner, B.J. Ryan, J. Spira, N.B. Wachsmuth, R.C. Roach. AltitudeOmics: The Integrative Physiology of Human Acclimatization to Hypobaric Hypoxia and Its Retention Upon Reascent. *PLOSone* 9(3):e92191, 2014. PMCID: PMC3962396
5. Foster, G.E., P.N. Ainslie, M. Stembridge, T.A. Day, A. Bakker, S.J. Lucas, N.C. Lewis, K.R. Burgess, D. MacLeod, **A.T. Lovering.** Pulmonary hemodynamics and shunting: a comparison of sea-level inhabitants to high altitude Sherpas. *J Physiol* 592(6): 1397-1409, 2014. PMID: 24396057

Additional recent publications of importance to the field (in chronological order)

1. Amann, M., M.W. Eldridge, **A.T. Lovering**, M.K. Stickland, D.F. Pegelow and J.A. Dempsey. Arterial oxygen content influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue. *J Physiol* 575(3):937-952, 2006. PMID:16793898, PMCID: PMC1995675
2. Romer, L.M., M. Amann, H.C. Haverkamp, **A.T. Lovering**, D.F. Pegelow and J.A. Dempsey. Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans *Am J Physiol:Regul Integr Comp Physiol* 292(1):R598-606, 2007. PMID: 16959862
3. **Lovering, A.T.**, L.M. Romer, H.C. Haverkamp, D.F. Pegelow, J.S. Hokanson and M.W. Eldridge. Intrapulmonary shunting and pulmonary gas exchange during normoxic and hypoxic exercise in healthy humans. *J Appl Physiol* 104:1418-1425, 2008. PMID: 18292301
4. Laurie, S.S., X. Yang, J.E. Elliott, K.M. Beasley & **A.T. Lovering**. Hypoxia-induced intrapulmonary arteriovenous shunting at rest in healthy humans *J Appl Physiol* 109:1072-1079, 2010. PMID: 20689088
5. Elliott, J.E., Y. Choi, S.S. Laurie, X. Yang, I.M. Gladstone & **A.T. Lovering.** Gas bubble composition does not affect detection of inducible intrapulmonary arteriovenous shunt at rest or during exercise in normoxia, hypoxia or hyperoxia. *J Appl Physiol* 110: 35-45, 2011. PMID: 20847134
6. **Lovering, A.T.**, M.K. Stickland, M. Amann, J.S. Hokanson and M.W. Eldridge. Effect of a patent foramen ovale on pulmonary gas exchange during normoxic and hypoxic exercise. *J Appl Physiol* 110: 1354–1361, 2011. PMID: 21372097, PMCID: PMC3290103
7. Laurie, S.S., J.E. Elliott, R.D. Goodman, **A.T. Lovering**. Catecholamine-induced intrapulmonary arteriovenous shunting in healthy humans at rest. *J Appl Physiol 113(8): 1213-1222, 2012*. PMID:22858627

8. Amann, M., S. Goodall, R. Twomey, A.W. Subudhi, **A.T. Lovering**, R.C. Roach. AltitudeOmics: On the consequences of high altitude acclimatization for the development of fatigue during locomotor exercise in humans *J Appl Physiol* 115(5): 634-42, 2013. PMID:23813531
9. Goodall, S. M., R. Twomey, M. Amann, E.Z. Ross, **A.T. Lovering**, Lee M. Romer, A.W. Subudhi, R.C. Roach. AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude. *Acta Physiol* 210 (4): 875–888, 2014. PMID: 24450855
10. HC Norris, TS Mangum, JW, Duke, TB Straley, RD Goodman, JA Hawn & **A.T. Lovering**. Exercise- and hypoxia-induced blood flow through intrapulmonary arteriovenous anastomoses is reduced in older adults. *J Appl Physiol 2014 doi:10.1152/japplphysiol.01125.2013* ***(In Press)***

D. Research Support

Ongoing Research Support

AHA Scientists Development Grant — Lovering (PI) — 07/01/2009 – 06/30/2014
GRANT # 2280238
Title: Cardiopulmonary responses to exercise & hypoxia in adult survivors of Bronchopulmonary Dysplasia
Major Goals: To determine the cardiopulmonary responses to exercise and hypoxia stress in full term, preterm and preterm subjects with BPD.
Role: PI

Defense Medical Research & Development Program — Lovering (PI) — 10/01/2010 – 09/30/2014
GRANT # W81XWH-10-2-0114/#DM1027581 JTCG5 TATRC
Title: Prediction of susceptibility to acute mountain sickness using hypoxia-induced intrapulmonary arteriovenous shunt and intracardiac shunt fractions
Major Goals: Develop a method to predict susceptibility of healthy humans to acute mountain sickness.
Role: PI

AHA Predoctoral Fellowship — Elliott (PI) — 07/01/2012 – 06/30/2014
Title: Epinephrine-induced recruitment of intrapulmonary arteriovenous shunt in healthy humans at rest
Major Goals: To determine the role of epinephrine in opening intrapulmonary shunts at rest and their role in gas exchange efficiency
Role: Mentor

Evonuk Memorial Graduate Fellowship in Environ Physiol — Elliott (PI) — 2013 – 2014
Title: Mechanisms of pulmonary gas exchange efficiency: Revisiting the paradigm
Major Goals: Demonstrate that blood flow through IPAVA provides a source of venous admixture that impairs pulmonary gas exchange efficiency.
Role: Mentor

Med. Research Foundation of OR Early Clinical Investigator — Duke (PI) — 12/01/2013 – 11/30/2014
GRANT # 1348
Title: Mitigation of cardiopulmonary sequelae associated with bronchopulmonary dysplasia
Major Goals: 1) Identify an excessive work of breathing as a limitation to exercise performance in adults with a history of extreme preterm birth (<30 weeks gestational age). 2) Demonstrate that exercise-induced pulmonary capillary hemorrhage occurs in adults with a history of extreme preterm birth (<30 weeks gestational age).
Role: Mentor

Completed Research Support

PeaceHealth Translational Research Award Program — Lovering (PI) — 07/01/2010 – 06/30/2011
GRANT # 429234
Title: Oxygen mediation of intrapulmonary arteriovenous anastomoses in healthy humans.
Major Goals: quantify shunt fraction under a variety of physiologic conditions in healthy humans.
Role: PI

Principle Investigator (**Lovering, Andrew Thomas**):

American Thoracic Society/American Lung Association Lovering (PI) 09/01/2010 – 08/31/2012
GRANT #C-10-014
Title: Prevention of intrapulmonary arteriovenous shunting in patients with COPD
Major Goals: Determine the role of arterial desaturation in the regulation of intrapulmonary arteriovenous pathways in subjects with COPD
Role: PI

Alberta Health Services Emerging Research Teams Grant Thébaud (PI) 10/01/2009 – 09/30/2013
GRANT # RES0002582
Title: Cardio-respiratory function, school age abilities and quality of life in extremely low birth weight infants.
Major Goals: Determine the long-term cardiopulmonary outcomes of children born prematurely.
Role: Co-I

Evonuk Memorial Graduate Fellowship in Environ Physiol Laurie (PI) 2010 – 2011
Title: Intrapulmonary Arteriovenous Anastomoses Contribute to Pulmonary Gas Exchange Inefficiency during Exercise in Healthy Humans.
Major Goals: Develop a refined nuclear medicine technique to quantify intrapulmonary shunt fraction
Role: Mentor

Defense University Research Instrumentation Program (DURIP) Halliwill (PI) 06/15/2011 – 06/14/2012
Title: Assessment of blood flow and perfusion during challenges to homeostasis in humans
Major Goals: To purchase a transcranial Doppler system and a Near Infrared Spectroscopy System
Role Co-PI

Defense University Research Instrumentation Program (DURIP) Lovering (PI) 06/15/2012 – 06/14/2013
Title: Multidimensional ultrasound assessment of blood flow & perfusion during challenges to homeostasis in humans
Major Goals: To purchase a Philips ie33 3D Doppler Ultrasound System
Role: PI

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED 07/30/2014

Applicant Identifier 150161

3. DATE RECEIVED BY STATE

State Application Identifier

1. * TYPE OF SUBMISSION

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a Federal Identifier

b. Agency Routing Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:** 0410963140000

* Legal Name: University of Colorado Denver

Department: 20353 -- SOM-EM MED CLINICAL | Division:

* Street1: Mail Stop F428, Anschutz Medical Campus | Street2: Building 500, 13001 East 17th Place, Room W1126

* City: Aurora | County: Adams | * State: CO: Colorado

Province: | * Country: USA: UNITED STATES | * ZIP / Postal Code: 80045-2571

Person to be contacted on matters involving this application

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
	Ryan	Anthony	Holland	

* Phone Number: 303-724-0090 | Fax Number: 303-724-0814 | Email: xenia@ucdenver.edu

6. * EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)*: 846000555

7. * TYPE OF APPLICANT H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type

❍ Women Owned ❍ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION: ● New

❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration

❍ D. Decrease Duration ❍ E. Other *(specify)*:

* Is this application being submitted to other agencies? ❍ Yes ● No

What other Agencies?

9. * NAME OF FEDERAL AGENCY: Dept. of the Army -- USAMRAA

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: 12.420

TITLE: Military Medical Research and Development

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Three New Ideas to Protect Special Forces from the Stress of High Altitude

12. PROPOSED PROJECT:

* Start Date	* Ending Date
12/01/2014	11/30/2016

13. CONGRESSIONAL DISTRICTS OF APPLICANT CO-006

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Dr.	Robert	Corwine	Roach Jr.	

Position/Title: Associate Professor | * Organization Name: University of Colorado Denver

Department: 20353 -- SOM-EM MED CLINICAL | Division:

* Street1: 12469 E 17th PL | Street2:

* City: Aurora | County: | * State: CO: Colorado

Province: | * Country: USA: UNITED STATES | * ZIP / Postal Code: 80045

* Phone Number: 303-724-1671 | Fax Number: | * Email: robert.roach@ucdenver.edu

15. ESTIMATED PROJECT FUNDING

a. * Total Federal Funds Requested	$699,499.00
b. * Total Non-Federal Funds	$0.00
c. * Total Federal & Non-Federal Funds	$699,499.00
d. * Estimated Program Income	$0.00

16* IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or other Explanatory Documentation

File Name:

19. Authorized Representative

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
	Ryan	Anthony	Holland	

* Position/Title: PreAward Manager
* Organization Name: University of Colorado Denver
Department: 60067 -- ADM VCR OGC ADMINISTR
Division:
* Street1: Mail Stop F428, Anschutz Medical Campus
Street2: Building 500, 13001 East 17th Place, Room W1126
* City: Aurora
County:
* State: CO: Colorado
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 80045-2571
* Phone Number: 303-724-0090
Fax Number: 303-724-0814
* Email: xenia@ucdenver.edu

*** Signature of Authorized Representative**	*** Date Signed**
Ryan Anthony Holland	07/30/2014

20. Pre-application File Name: Pre_Application.pdf Mime Type: MIMETYPE

15 May 2014

PRE-PROPOSAL FOR

Three New Ideas to Protect Special Forces from the Stress of High Altitude

Research Areas of Interest

3. Force Health Protection and Environmental Medicine
a. Optimal Acclimatization Strategy
b. High Altitude Pulmonary Edema/High Altitude Cerebral Edema

Prepared By

Robert C. Roach, Ph.D.

Altitude Research Center, University of Colorado Denver
Mail Stop F-524, 12469 E. 17th Place
Aurora, Co 80045
Phone: 303-724-1770
Fax: 303-724-1660

Keywords: high-altitude, AMS, HAPE, HACE, acclimatization, edema, hypoxia, nifedipine, methazolamide, quercetin, metformin, nutraceutical

1. Scope/Introduction. (limit: 20 lines of text)

High altitude illnesses pose a significant threat to the warfighter rapidly exposed to high altitudes.[1] Unfortunately, no major advances have been made in promoting acclimatization or preventing high-altitude illnesses in the last 25 years. **We propose to test three novel ideas to rapidly advance warfighter performance in a state-of-the-art field study.**

In other recent DoD-funded studies we have been very productive. We have completed the first ever test of a gene-based prediction of high-altitude illness, and we have completed the first molecular and cellular biology study of how humans adjust to hypoxia.[2] Additionally, our colleagues have completed DARPA-funded work to screen hundreds of compounds for effectiveness in preventing high altitude pulmonary and cerebral edema. Each of these studies contributes to the novel ideas presented here. **Thus, our team is currently the best-suited civilian or military team in the world to address the scientific medical concerns of US Special Forces related to high altitude.**

To improve high-altitude performance we will test: **quercetin, nifedipine + methazolamide and metformin.** Quercetin, an over-the-counter nutraceutical, and nifedipine+methazlamide, two drugs already approved for use in humans, are effective for preventing high-altitude cerebral and pulmonary edema. Metformin, a drug commonly used to treat diabetes, induces biochemical changes recently linked to successful acclimatization and protection from high altitude illnesses. **We propose one or more of these approaches will substantially improve warfighter performance in the high altitude environment.**

2. Background Information and Scientific Approach. (limit: 2 pages of text)

This proposal directly addresses: *3. Force Health Protection and Environmental Medicine: a. Optimal Acclimatization Strategy; and b. High Altitude Pulmonary Edema/High Altitude Cerebral Edema*.

SOCCOM understands the importance of protecting SOF warfighters from the challenges of high-altitude illness, so the rationale for these experiments is brief. Acute mountain sickness (AMS) can cause debilitating symptoms—the PI has personally observed a Marine Corps Force Recon operator give up command of his unit on a training exercise due to symptoms of AMS incurred on rapid insertion to 14,000 ft. High-altitude pulmonary edema (HAPE) and high-altitude cerebral edema (HACE) can kill.[1] Although HAPE/HACE incidence is low, their impact can be great. For example, if a warfighter develops HAPE or HACE, immediate evacuation may be necessary. Such a diversion raises potential catastrophic risks for other troops. In support of the cost and risk of high-altitude illnesses is that during a major combat operation in Afghanistan, ~12% of medevacs and hospital admissions were due to severe AMS/HAPE/HACE. **This proposal directly addresses the need of SOF warfighters to expand their arsenal of options when working at high altitudes.**

We understand SOCCOM is interested in solutions, today. Therefore we have devised an experiment to test three different compounds for advancing acclimatization and preventing high-altitude illnesses. **Each can be tested today. If successful, any of the three could be used by SOF warfighters tomorrow.**

Next we outline why each of these three compounds shows promise for improving performance and preventing high-altitude illnesses. Then we briefly describe our experimental approach.

What is quercetin and why might it work to aid acclimatization and prevent high-altitude illnesses? Quercetin is an antioxidant and an anti-inflammatory agent, widely present in fruits and vegetables.[3] It has been reported to possess antioxidant effects as free radical scavenger, hydrogen-donating compound, a singlet oxygen quencher, and metaloid chelator. Quercetin can also reduce inflammation by attenuation of the redox-sensitive transcription factor and of scavenging free radicals. **We think quercetin will be effective for preventing high-altitude illnesses because 1) animal studies have shown it to be very effective in preventing cerebral edema,[4,5] and 2) it acts by suppressing inflammation.** We have recently shown in human that lower levels of inflammation are associated with protection from acute mountain sickness.[6] And dexamethasone, a potent anti-inflammatory steroid, is the most potent drug known for preventing and treating AMS, HAPE and HACE. In quercetin treated rats, each dose of quercetin reduced brain water content and transvascular leakage to near normoxia values, comparable to the protection afforded by dexamethasone. Although dexamethasone is extremely effective in preventing and treating high-altitude illnesses, its safety profile is troublesome. Thus, an alternative to dexamethasone is desirable. We think quercetin fits the role for a potential dexamethasone replacement, and it is attractive as a safe, over-the-counter medication that could be used by SOF warfighters immediately.

What is nifedipine+methazolamide, and why might it work to prevent high-altitude illnesses? Nifedipine is a proven to prevent HAPE by lowering pulmonary artery pressure.[1] Methazolamide is a cousin of the widely used and successful AMS/HAPE/HACE preventing drug acetazolamide (Diamox).[1,7,8] The advantages of methazolamide are that lower doses with fewer side effects seem to achieve equivalent protective effects in animal and human studies. **Why consider these two drugs used in combination?** Our colleague Dr. Dave Irwin recently showed that the combination of nifedipine + methazolamide was the most effective of all compounds besides dexamethasone in preventing HAPE and HACE in a rat model of these diseases. This combination of two FDA approved drugs has never been tested in humans. Based on the animals studies by Dr. Irwin and the effectiveness of each drug alone in humans **we think the combination of nifedipine + methazolamide has considerable potential to be effective in protecting humans from all the high-altitude illnesses.**

What is metformin, and why might it work to aid acclimatization and prevent high-altitude illnesses? Working together with Yang Xia at UT Houston we had the opportunity to study oxygen transport modulation during human acclimatization to high altitude. Since acclimatized humans are nearly completely protected from high-altitude illness and have better physical and cognitive function we reasoned that any factor responsible for enabling acclimatization would potentially play a role in invoking acclimatization–like responses in altitude-naïve subjects, such as warfighters being rapidly deployed to high altitude.

To function effectively in O_2 uptake and release, erythrocytes rely on sophisticated regulation of hemoglobin (Hb)-O_2 affinity by allosteric modulators. One of the best-known allosteric modulators is 2,3-bisphophosphoglycerate (2,3-BPG). Earlier studies demonstrated that erythrocyte 2,3-BPG levels are elevated at a high altitude and in sickle cell disease.[9] However, factors responsible for 2,3-BPG induction at high altitude are unknown. To address this question, we tested 21 individuals placed at high altitude for different periods of time. We found that: 1)

Plasma adenosine and erythrocyte 2,3-BPG levels were significantly elevated in normal individuals following 24 hours at high altitude compared to sea level. 2) Adenosine and 2,3-BPG levels were further enhanced after 16 days at high altitude. 3) Elevated circulating adenosine levels significantly correlated with increased erythrocyte 2,3-DPG levels in normal individuals at high altitude. Next, we found *in vitro* evidence that adenosine signaling via A_{2B} adenosine receptors (ADORA2B) induced 2,3-BPG production in a protein kinase A (PKA) and AMP activated protein kinase (AMPK)-dependent manner in cultured human erythrocytes. These studies show that adenosine signaling through ADORA2B regulates erythrocyte 2,3-BPG induction as a function of altitude and identifies new targets to enhance O_2 release under hypoxia conditions.

Metformin is an FDA approved drug to treat diabetic patients that decreases hyperglycemia primarily by suppressing glucose production by the liver.[10] Although the molecular basis of metformin is not fully understood, it is well known to activate AMPK and PKA. Based on our initial observation that PKA and AMPK are essential to regulate 2,3-BPG induction and promote O_2 release from erythrocytes, we propose to test the intriguing possibility that metformin is a safe drug to induce O_2 release and thereby mimic acclimatization and and prevent high-altitude sickness.

Overall Study Approach

We will test 60 young men and women resembling young, fit military recruits for the effectiveness of three compounds versus placebo (15 in each group). Each compound has been selected for strong scientific rationale of potential effectiveness and immediacy of availability for use by the SOF warfighter operating at high altitudes. Human studies are difficult, expensive and time consuming. Our team has developed considerable expertise in this area and can deliver a thorough evaluation of these compounds with the goal of rapidly improving SOCCOM options for dealing with high altitude medical problems.

3. Bibliography. (limit: list of 10 documents)

1. Hackett PH, Roach RC. Current concepts: High-altitude illness. The New England Journal of Medicine 2001;345:107-14.
2. Subudhi AW, Bourdillon N, Bucher J, et al. AltitudeOmics: The Integrative Physiology of Human Acclimatization to Hypobaric Hypoxia and Its Retention upon Reascent. PLoS One 2014;9:e92191.
3. Dajas F, Andres AC, Florencia A, Carolina E, Felicia RM. Neuroprotective actions of flavones and flavonols: mechanisms and relationship to flavonoid structural features. Central nervous system agents in medicinal chemistry 2013;13:30-5.
4. Patir H, Sarada SKS, Singh S, Mathew T, Singh B, Bansal A. Quercetin as a prophylactic measure against high altitude cerebral edema. Free radical biology & medicine 2012;53:659-68.
5. Prasad J, Baitharu I, Sharma AK, Dutta R, Prasad D, Singh SB. Quercetin reverses hypobaric hypoxia-induced hippocampal neurodegeneration and improves memory function in the rat. High altitude medicine & biology 2013;14:383-94.
6. Julian CG, Subudhi AW, Wilson MJ, Dimmen AC, Pecha T, Roach RC. Acute mountain sickness, inflammation, and permeability: new insights from a blood biomarker study. Journal of Applied Physiology 2011;111:392-9.

7. Imray C, Wright A, Subudhi A, Roach R. Acute mountain sickness: pathophysiology, prevention, and treatment. Progress in cardiovascular diseases 2010;52:467-84.
8. Lisk C, McCord J, Bose S, et al. Nrf2 activation: a potential strategy for the prevention of acute mountain sickness. Free Radic Biol Med 2013;63:264-73.
9. Zhang Y, Dai Y, Wen J, et al. Detrimental effects of adenosine signaling in sickle cell disease. Nature medicine 2011;17:79-86.
10. Hardie DG. AMPK: a target for drugs and natural products with effects on both diabetes and cancer. Diabetes 2013;62:2164-72.

4. Goals and/or Objectives. (limit: 20 lines of text)

Goal 1: Evaluate effectiveness for preventing AMS, HAPE and HACE of quercetin, nifedipine+methazolamide and metformin compared to placebo during a series of tests of performance at high altitude.

Objective 1. Evaluate effectiveness of the neutraceutical quercetin to prevent high altitude illness, and improve physical and cognitive performance upon rapid ascent and over three days at altitudes between 10,000 and 13,000 feet.

Objective 2. Evaluate a comnbination of nifedipine+methazoalmide, FDA approved drugs that have been shown alone to help with HAPE (nifedipine) and AMS/HACE (methazolamide), to prevent high-altitude illness, and improve physical and cognitive performance upon rapid ascent and over three days at altitudes between 10,000 and 13,000 feet.

5. Work. (limit: one page of text)

Work to be accomplished will include:

1. Obtain University of Colorado and Oregon IRB approval for study.
2. Obtain DOD HRPO approval for study.
3. Recruit 60 volunteers from University of Oregon community at sea level. All subjects will pass rigorous physical screening, including mandatory passage of Army PFT at age-specific levels or SOCCOM directed Special Forces fitness assessment.
4. Carefully screen Oregon volunteers for inclusion/exclusion criteria.
5. Select Oregon and Colorado trails with similar elevation gain over three miles.
6. Schedule Oregon volunteers for trip to Colorado weekends in Colorado.
7. Establish Colorado basecamp in Breckenridge, Colorado.
8. Conduct test weekend with Oregon staff in Colorado to review and revise all pertinent procedures.
9. Begin serial weekend testing for 5 to 10 weekends over 12 weeks, with 10-20 subjects per group.
10. Conduct daily data analysis and interpretation of AMS, physical performance and cognitive function data.
11. Finish series of weekend tests.
12. Complete data entry, prepare for data analysis.

13. Break drug code and discover which of the proposed compounds had a substantial effect on AMS, HAPE and HACE prevention, physical performance or cognitive performance in real world field conditions at high altitude compared to placebo.

This ambitious plan is fully realizable because of our recent experience conducting three DoD-TATRC-funded field studies. In those study we tested whether a test of gene expression at sea level could predict who would later develop AMS. In the first of two field trials for that study we could predict 9/10 young, healthy civilians who would either get sick with AMS or stay healthy. The validation of that study was just completed and analyses are underway. If successful, a test will be developed for broad military and civilian use to predict at sea level susceptibility of AMS. The third study was a massive effort to study 21 subjects at sea level and after several weeks at high altitude at 17,200 feet in Bolivia. The results from that study are the first description of the molecular mechanisms responsible for the human body's response to prolonged hypoxia and have led us to propose metformin as a novel candidate drug for improving human performance at high altitude. We conducted both of those studies at the same time, managed to get IRB and HRPO clearance for both in a reasonably timely manner, and have met all milestones for the proposed studies. This success gives a great confidence that we can complete the proposed studies as outlined here.

6. Deliverables. (limit: 20 lines of text)
We will deliver interim reports every quarter on study progress. At the completion of 18 months, we will deliver preliminary results form the field trials. At 24 months we will present a final report including analysis of all results from all trials, a presentation suitable for wide distribution of the final results, and executive recommendations for implementations of the findings for immediate SOCCOM application.

7. Government Furnished Property. (limit: 20 lines of text)
Not applicable.

8. Place of Performance. (limit: 5 lines of text)
University of Colorado Anschutz Medical Campus, Aurora, Colorado; field basecamp in Breckenridge, Colorado; and University of Oregon, Department of Human Physiology, Eugene, Oregon.

9. Period of Performance. (limit: 5 lines of text)
24 months. Six months for project startup and IRB + HRPO approvals, 12 months for field data collection, 6 months for data analysis and report writing. This schedule allows time for schedule slippage if DoD HRPO is slow in processing IRB approval, and allows ample time for our experienced team to recruit subjects, collect data in the field and analyze the data for efficacy in preventing high altitude illness and improving physical and cognitive performance.

10. Human Use and Animal Use. (limit: 20 lines of text)
We will conduct human experiments, similar in nature to our recent DoD-funded and HRPO approved field studies in South America and in Breckenridge, Colorado. We have a template in place of HRPO approved procedures and protocols for every aspect of the proposed studies except the specific risks of the proposed compounds. Furthermore, if we are selected to present a

full proposal we will simultaneously prepare an IRB submission for presentation to our local IRB on the day we receive notification of funding. Since this will be nearly identical to previously approved studies we expect to have local IRB approval within three months of notification of funding, and HRPO approval hopefully within three to six months of submission to HRPO. Since we will start the IRB process on ‘notification of funding’, which is likely several months before ‘start of funding’, we will gain an extra few critical months on the funded timeline to allow for idiosyncrasies in HRPO processing.

11. Principal Investigator (PI) and Support Personnel. (limit: 20 lines of text to list personnel and their contact information)

Robert Roach, PhD. Principal Investigator, Director, Altitude Research Center, University of Colorado Anschutz Medical Campus, rroach@hypoxia.net

Andrew Subudhi, Ph.D. Co-Principal Investigator. Jointly responsible with PI for overall conduct of the study. Associate Professor, University of Colorado, Colorado Springs, asubudhi@uccs.edu

Andrew Lovering, Ph.D., Co-Investigator, responsible for subject recruitment, screening and management. Associate Professor, Department of Human Performance, University of Oregon, lovering@uoregon.edu

Yang Xia, MD, PhD, Co-Investigator. Responsible for analysis and interpretation of metformin results in comparison with findings in animal and other human studies of adenosine and acclimatization, yang.xia@uth.tmc.edu

David Irwin, PhD, Consultant on translation of DARPA-funded drug discovery for protection from high altitude illness from rodents to humans, Department of Medicine, University of Colorado Anschutz Medical Campus, David.Irwin@ucdenver.edu

Mr. Rod Alne, US Air Force CMSgt Pararescue, Ret, a former Air Force pararescue specialist with extensive SOF and high altitude experience will serve as our special forces applications advisor, rod.alne@thepeakinc.com

12. Budget. (limit: 20 lines of text)

Direct Cost University of Colorado Denver: These costs will include all field logistics, room rental, food and transport costs both local in Colorado and from Oregon to Colorado. Colorado staff will be primarily responsible for the Colorado IRB and DOD HRPO IRB document preparation. Colorado staff will be primarily responsible for all DOD reporting. Colorado staff will be primarily responsible for all data entry and analysis, and final interpretation and recommendations to the DOD.

Subcontract University of Oregon. This will include staff to recruit, screen and pre-test 100 subjects to get a clean pool of 60 research subjects who can pass all inclusion/exclusion criteria, including the modified Army PFT. The Oregon budget will also include support for the Oregon IRB submission. Subject payments will also be made by University of Oregon.

	Year 1	Year 2	Grand Total
Direct Costs UC Denver	$ 195,000	$ 170,000	$ 365,000
Subcontract U of O	$ 90,000	$ 35,000	$ 125,000
Total Direct Costs	$ 285,000	$ 200,000	$ 485,000
Total Indirect Costs	$ 121,000	$ 93,500	$ 214,500
Grand total	$ 406,000	$ 293,500	$ 699,500

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Robert C. Roach, Ph.D.	Associate Professor
eRA COMMONS USER NAME ROACH.R	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The Evergreen State College, Olympia, WA	B.S.	1979	Biochemistry
Cornell University, Ithaca, NY	M.S.	1985	Nutritional Science
University of New Mexico, Albuquerque, NM	Ph.D.	1994	Exercise Physiology

A. Personal Statement

My research focuses on the broad area of human responses to hypoxia. Current research is focused on three major areas: cerebrovascular hemodynamics in hypoxia and exercise; transcriptomic prediction of human responses to hypoxia; and the integration of systems biology with physiology to understand the molecular and cellular mechanisms of oxygen sensing in humans. We have recently shown that hypoxia impairs cerebral autoregulation; research is underway to begin to understand the importance of this finding and its possible mechanisms. We found that a gene expression signature from a blood sample collected in Denver predicted >95% of those who later developed acute mountain sickness. A recent validation study at lower altitudes in a more diverse population confirmed these findings. Studies are underway to examine the physiological links of these transcriptomic markers and pathways that lead to susceptibility to altitude illness. And finally, we are undertaking studies to link a comprehensive 'omics' pathway of oxygen sensing (transcriptomics, epigenetics, proteomics, metabolomics) to physiological responses that serve to improve oxygen transport during acute and chronic hypoxia. In my position as Research Director and now Director of the Altitude Research Center I have mentored 17 researchers, ranging from research fellows in Emergency Medicine, Neurology and Pulmonary Medicine to medical students, postdoctoral fellows and undergraduate students. I am active on the Department of Emergency Medicine research council, and mentor for an additional six junior faculty on routine research-related topics.

B. Positions and Honors

Positions and Employment

2010-present	Director, Altitude Research Center, University of Colorado Denver, Denver, CO
2003-2010	Associate Director and Chief, Research Division, Altitude Research Center UCDHSC, Denver, CO
2001-2003	Scientist, New Mexico Resonance, Albuquerque, NM
1999-present	Co-Chairman, International Hypoxia Symposia (www.hypoxia.net)
1999-2002	Clinical Assistant Professor, Department Surgery, Div Emergency Medicine, UCDHSC,O
1999-2003	Clinical Assistant Professor, Department Medicine, University of New Mexico, Albuquerque, NM
1999-2000	Research Assistant Professor, Department Life Sciences, New Mexico Highlands University, Las Vegas, NM
1998-1999	Visiting Professor, Department of Life Sciences, New Mexico Highlands University, Las Vegas, NM
1996-1998	Alfred Benzon Research Fellow, Copenhagen Muscle Research Center, Copenhagen, Denmark.
1994-1996	Associate Scientist, Cardiopulmonary Physiology, Institute Basic Applied Medical Research, The Lovelace Institutes, Albuquerque, NM
1993-2005	Consultant, Life Support Systems, Odyssey Around the World Balloon Flight, Albuquerque, NM
1990-1994	Research Physiologist, Oxygen Transport Program, The Lovelace Institutes, Albuquerque, NM
1989-2005	Associate Scientist, Siberian-Alaskan Medical Research Exchange, Section Cold Altitude Physiology, University of Alaska, Anchorage, AK

1982-1990 Associate Director, Denali Medical Research Project, University of Alaska, Anchorage, AK

Professional Memberships

American Physiological Society; American College of Sports Medicine; American Association for the Advancement of Science; American Alpine Club; International Society for Mountain Medicine

Review and Referee Work

Appointed, Editorial Board, Journal of Applied Physiology, 2006 to present
Appointed, Editorial Board, Medicine Science Sports and Exercise, 2005 to 2011
Appointed, Section Editor, Hypoxia and High Altitude, Extreme Medicine and Physiology, BMC Journals, 2011-present.
Invited Reviewer, DOD Brain Injury Study Section, American Institute of Biological Science, 2009-2010.

Honors and Awards

Elected Fellow, American College of Sports Medicine (FACSM), fall 2004.
Appointed, American Physiological Society Porter Scholarship Selection Committee, 2005-2008
Appointed, American College of Sports Medicine, Constitution, Bylaws and Operating Codes Committee, 2006-2009
Appointed, American College of Sports Medicine, Promotions and Fellowship Committee, 2011 to present

C. Selected Peer-Reviewed Publications (selected from 86 peer-reviewed publications)
Most relevant to the current application

Roach EB, Bleiberg J, Lathan CE, Wolpert L, Tsao JW, **Roach R.C.**, AltitudeOmics: Decreased reaction time after high altitude cognitive testing is a sensitive metric of hypoxic impairment. Neuroreport. 2014 Apr 9

Subudhi, A., J. Bucher, N. Bourdillon, C. Davis, J. Elliott, M. Eutermoster, O. Evero, J.L. Fan, S. Jameson-Van Houten, C.G. Julian, J. Kark, S. Kark, B. Kayser, J.P. Kern, S.E. Kim, C. Lathan, S.S. Laurie, A.T. Lovering, R. Paterson, D. Polaner, B.J. Ryan, J. Spira, N.B. Wachsmuth, and **Roach R.C.**, AltitudeOmics: The Integrative Physiology of the Onset and Retention of Acclimatization to Hypoxia in Humans. PLoS One, 2014; 9(3).

Roach R.C., Wagner PD, Hackett PH Translation in progress: hypoxia. J Appl Physiol (1985). 2014 Apr 1;116(7)

Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness Journal of Applied Physiology, 2014; 117(7).

Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Effect of ascent and acclimatization to 5,260 m on regional cerebral oxygen delivery Experimental Physiology, 2014.

Julian, C.G., A.W. Subudhi, R.C. Hill, M.J. Wilson, A.C. Dimmen, K.C. Hansen, and **R.C. Roach**, Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. Journal of Applied Physiology, 2013; 116(7).

Goodall, S., M. Amann, R. Twomey, A.W. Subudhi, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude Acta Physiologica, 2014; 210(4).

Fan, J.L., A. Subudhi, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and **R.C. Roach**, AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high altitude acclimatisation and re-exposure Journal of Applied Physiology, 2014; 116(7).

Davis, C., E. Reno, B. Vestal, E. Maa, and **R.C. Roach**, Risk Factors for Headache While Hiking Above 4300 Meters. High Altitude Medicine and Biology (in review), 2013.

Wilson MJ, Julian CG, **Roach R.C**. Genomic analysis of high altitude adaptation: innovations and implications. Curr Sports Med Rep 2011;10:59-61.

Subudhi AW, Dimmen AC, Julian CG, Wilson MJ, Panerai RB, **Roach R.C**. Effects of acetazolamide and dexamethasone on cerebral hemodynamics in hypoxia. J Appl Physiol 2011;110:1219-25.

Roach R.C., Kayser B, Hackett P. Pro: Headache should be a required symptom for the diagnosis of acute mountain sickness. High Alt Med Biol 2011;12:21-2.

Julian CG, Subudhi AW, Wilson MJ, Dimmen AC, Pecha T, **Roach R.C**. Acute Mountain Sickness,

Inflammation and Permeability: New Insights from a Blood Biomarker Study. J Appl Physiol 2011.
Subudhi AW, Panerai RB, **Roach R.C**. Effects of Hypobaric Hypoxia on Cerebral Autoregulation. Stroke 2010.
Subudhi AW, Panerai RB, **Roach R.C**. Acute hypoxia impairs dynamic cerebral autoregulation: results from two independent techniques. J Appl Physiol 2009;107:1165-71.
Subudhi AW, Miramon BR, Granger ME, **Roach R.C**. Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. J Appl Physiol 2009;106:1153-8.
Loeppky JA, Icenogle MV, Charlton GA, Conn CA, Maes D, Riboni K, Gates L, Melo MFV, **Roach R.C**. Hypoxemia and acute mountain sickness: which comes first? High Alt Med Biol 2009;9:271-9.

<u>Additional recent publications of importance to the field</u>

Ezzati M, Horwitz MEM, Thomas DSK, Friedman AB, **Roach RC**, Clark T, Murray CJL, Honigman B. Altitude, life expectancy and mortality from ischaemic heart disease, stroke, COPD and cancers: national population-based analysis of US counties. Journal Epidemiology Community Health 2011.
Browne VA, Toledo-Jaldin L, Davila RD, Lopez LP, Yamashiro H, Cioffi-Ragan D, Julian CG, Wilson MJ, Bigham AW, Shriver MD, Honigman B, Vargas E, **Roach RC**, Moore LG. High-end arteriolar resistance limits uterine artery blood flow and restricts fetal growth in preeclampsia and gestational hypertension at high altitude. Am J Physiol Regul Integr Comp Physiol 2011;300:R1221-9.
Asgari S, Subudhi AW, **Roach RC**, Liebeskind DS, Bergsneider M, Hu X. An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. J Neurosci Methods 2011;197:171-9.
Roach RC. Hypoxia and the Circulation. New York: Springer; 2007.
Hackett PH, **Roach RC**. High Altitude Medicine. In: Auerbach PS, ed. Wilderness Medicine. Vol 6th. Philadelphia: Mosby Elsevier; 2011:2-36.
Subudhi AW, Panerai RB, **Roach RC**. Effects of Hypobaric Hypoxia on Cerebral Autoregulation. Stroke 2010.
Imray C, Wright A, Subudhi A, **Roach RC**. Acute mountain sickness: pathophysiology, prevention, and treatment. Progress in Cardiovascular Diseases 2010;52:467-84.
Tissot van Patot MC, Serkova NJ, Haschke M, Kominsky DJ, **Roach RC**, Christians U, Henthorn TK, Honigman B. Enhanced leukocyte HIF-1alpha and HIF-1 DNA binding in humans after rapid ascent to 4300 m. Free Radic Biol Med 2009;46:1551-7.
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Hu X, Subudhi AW, Xu P, Asgari S, **Roach RC**, Bergsneider M. Inferring cerebrovascular changes from latencies of systemic and intracranial pulses: a model-based latency subtraction algorithm. J Cereb Blood Flow Metab 2009;29:688-97.
Subudhi AW, Lorenz MC, Fulco CS, **Roach RC**. Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. Am J Physiol Heart Circ Physiol 2008;294:H164-71.
Firth PG, Zheng H, Windsor JS, Sutherland AI, Imray CH, Moore GWK, Semple JL, **Roach RC**, Salisbury RA. Mortality on Mount Everest, 1921-2006: descriptive study. BMJ 2008;337:a2654.
Subudhi AW, Dimmen AC, **Roach RC**. Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. J Appl Physiol 2007;103:177-83.
Roach RC. Hypoxia and Exercise. New York: Springer; 2005.
Loeppky JA, Icenogle MV, Maes D, Riboni K, Hinghofer-Szalkay H, **Roach RC**. Early fluid retention and severe acute mountain sickness. J Appl Physiol 2005;98:591-7.
Roach RC. Hypoxia Through the Lifecycle. New York: Kluwer Academic Plenum Publishers; 2003.
Møller K, Paulson OB, Hornbein TF, Colier WNJM, Paulson AS, **Roach RC**, Holm S, Knudsen GM. Unchanged cerebral blood flow and oxidative metabolism after acclimatization to high altitude. J Cereb Blood Flow Metab 2002;22:118-26.
Roach RC, Stepanek J, Hackett P. Acute Mountain Sickness And Cerebral Edema. (Rock P, Pandolf K, eds.). Washington, DC: US Army; 2002.
Bestle MH, Olsen NV, Poulsen TD, **Roach RC**, Fogh-Andersen N, Bie P. Prolonged hypobaric hypoxemia attenuates vasopressin secretion and renal response to osmostimulation in men. J Appl Physiol 2002;92:1911-22.

Hackett PH, **Roach RC**. High-altitude illness. N Engl J Med 2001;345:107-14.
Roach RC, Maes D, Sandoval D, Robergs RA, Icenogle M, Hinghofer-Szalkay H, Lium D, Loeppky JA. Exercise exacerbates acute mountain sickness at simulated high altitude. J Appl Physiol 2000;88:581-5.
Roach RC. Hypoxia: Into the Next Millenium. (Wagner P, Hackett P, eds.). NY: Plenum/Kluwer Academic; 1999.
Roach RC, Koskolou MD, Calbet JAL, Saltin B. Arterial O2 content and tension in regulation of cardiac output and leg blood flow during exercise in humans. Am J Physiol 1999;276:H438-45.
Roach RC, Greene ER, Schoene RB, Hackett PH. Arterial oxygen saturation for prediction of acute mountain sickness. Aviation, space, and environmental medicine 1998;69:1182-5.
Koskolou MD, **Roach RC**, Calbet JAL, Rådegran G, Saltin B. Cardiovascular responses to dynamic exercise with acute anemia in humans. Am J Physiol 1997;273:H1787-93.
Koskolou MD, Calbet JAL, Rådegran G, **Roach RC**. Hypoxia and the cardiovascular response to dynamic knee-extensor exercise. Am J Physiol 1997;272:H2655-63.
Roach RC, Loeppky JA, Icenogle MV. Acute mountain sickness: increased severity during simulated altitude compared with normobaric hypoxia. J Appl Physiol 1996;81:1908-10.
Loeppky JA, Scotto P, **Roach RC**. Acute ventilatory response to simulated altitude, normobaric hypoxia, and hypobaria. Aviation, space, and environmental medicine 1996;67:1019-22.
Honigman B, Theis MK, Koziol-McLain J, **Roach RC**, Yip R, Houston C, Moore LG, Pearce P. Acute mountain sickness in a general tourist population at moderate altitudes. Ann Intern Med 1993;118:587-92.
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Grissom CK, **Roach RC**, Sarnquist FH, Hackett PH. Acetazolamide in the treatment of acute mountain sickness: clinical efficacy and effect on gas exchange. Ann Intern Med 1992;116:461-5.
Schoene RB, Swenson ER, Pizzo CJ, Hackett PH, **Roach RC**, Mills WJ, Henderson WR, Martin TR. The lung at high altitude: bronchoalveolar lavage in acute mountain sickness and pulmonary edema. J Appl Physiol 1988;64:2605-13.
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Schoene RB, Hackett PH, Henderson WR, Sage EH, Chow M, **Roach RC**, Mills WJ, Martin TR. High-altitude pulmonary edema. Characteristics of lung lavage fluid. JAMA 1986;256:63-9.
Schoene RB, **Roach RC**, Hackett PH, Harrison G, Mills WJ. High altitude pulmonary edema and exercise at 4,400 meters on Mount McKinley. Effect of expiratory positive airway pressure. Chest 1985;87:330-3.
Roach RC, Larson EB, Hornbein TF, Houston CS, Bartlett S, Hardesty J, Johnson D, Perkins M. Acute mountain sickness, antacids, and ventilation during rapid, active ascent of Mount Rainier. Aviation, space, and environmental medicine 1983;54:397-401.
Larson EB, **Roach RC**, Schoene RB, Hornbein TF. Acute mountain sickness and acetazolamide. Clinical efficacy and effect on ventilation. JAMA 1982;248:328-32.

D. Ongoing Research Support

DMDRP W81XWH-11-2-0034 (Roach, PI) 12/20/2010-6/30/2014
<u>Prediction of acute mountain sickness using a blood-based test</u>
This project aims at developing a rapid, cost-effective, pre-ascent screening test to predict individual risk of acute mountain sickness (AMS) for military use.

DMDRP W81XWH-11-2-0040 (Roach, PI) 01/01/2011-6/30/2014
<u>AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude</u>
This project aims at advancing high-altitude medical research by discovering the basic molecular mechanisms of acclimatization that protect soldiers from high altitude illness.

DARPA (**PI: Irwin, D, Co-PI: Roach**) (01/01/2012-12/31/2014)
<u>Rapid Acclimatization to Hypoxia at Altitude</u>.
The advancement of high-altitude medical research by discovering novel preventive measures for acute mountain sickness.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Andrew W. Subudhi	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) asubudhi	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
The Colorado College	B.A.	1992	Mathematics
Colorado State University	M.S.	1996	Exercise Science
University of Utah	Ph.D.	2000	Exercise Physiology
University of Colorado Health Science Center	Post Doc	2003-5	Altitude Physiology

A. Personal Statement

My doctoral training in exercise physiology has given me a solid foundation for assessing cardiovascular, respiratory, and neuromuscular physiology in human subjects. During my PhD training, and for 5 years afterwards, my primary responsibility was monitoring training adaptations of athletes who were living and training at altitude in preparation for the 1998, 2002 and 2006 Olympic Winter Games. Based on my work in this area, I was invited to participate with research teams from Stanford University and the United States Army to study the physiological effects of acute altitude exposure and acclimatization. These experiences fueled my motivation to pursue a post-doctoral training under the direction of Dr. Robert Roach at the University of Colorado Altitude Research Center (ARC). While at ARC, I learned several techniques for assessing cerebrovascular physiology. Since securing a tenure-track position within the University of Colorado, I have developed a line of research investigating the influence of cerebral blood flow and oxygenation on health and performance at altitude. Given my breadth of knowledge and skill in assessing integrative physiological responses to hypoxia, I am particularly well prepared and suited for my role in this project.

B. Positions and Honors

<u>Positions</u>

1997 - 2005	Research Scientist, The Orthopedic Specialty Hospital (TOSH), Intermountain Health Care, Salt Lake City, UT.
2000 - 2008	Adjunct Assistant Professor, University of Utah, Division of Foods & Nutrition, Salt Lake City, UT.
2001 - 2005	Adjunct Assistant Professor, University of Utah, Dept. of Exercise & Sport Science, Salt Lake City, UT.
2005 - 2011	Assistant Professor, University of Colorado at Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2005 - 2011	Assistant Professor, University of Colorado at Denver, Dept. of Surgery, Denver, CO.
2011 - Present	Associate Professor, University of Colorado Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2011 - Present	Associate Professor, University of Colorado Denver/Anschutz Medical Campus, Dept. of Emergency Medicine, Denver, CO.

<u>Professional Memberships</u>

1995 – Present	Member of the American College of Sports Medicine
1996 – Present	Certified Strength and Conditioning Specialist (C.S.C.S.)
2000 – Present	Member of the American Physiological Society

C. Selected Peer-Reviewed Publications (selected from 43 peer-reviewed publications)

Most relevant to the current application

- Subudhi, A., J. Bucher, N. Bourdillon, C. Davis, J. Elliott, M. Eutermoster, O. Evero, J.L. Fan, S. Jameson-Van Houten, C.G. Julian, J. Kark, S. Kark, B. Kayser, J.P. Kern, S.E. Kim, C. Lathan, S.S. Laurie, A.T. Lovering, R. Paterson, D. Polaner, B.J. Ryan, J. Spira, N.B. Wachsmuth, and Roach R.C.(2014) AltitudeOmics: The Integrative Physiology of the Onset and Retention of Acclimatization to Hypoxia in Humans. PLoS One; 9(3).

- Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and R.C. Roach (2014) AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness Journal of Applied Physiology; 117(7).

- Subudhi, A., J.L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, A.T. Lovering, and R.C. Roach (2014) AltitudeOmics: Effect of ascent and acclimatization to 5,260 m on regional cerebral oxygen delivery Experimental Physiology.

- Julian, C.G., A.W. Subudhi, R.C. Hill, M.J. Wilson, A.C. Dimmen, K.C. Hansen, and R.C. Roach (2014) Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. Journal of Applied Physiology; 116(7).

- Goodall, S., M. Amann, R. Twomey, A.W. Subudhi, A.T. Lovering, and R.C. Roach, AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude Acta Physiologica, 2014; 210(4).

- Fan, J.L., A. Subudhi, O. Evero, N. Bourdillon, B. Kayser, A.T. Lovering, and R.C. Roach (2014) AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high altitude acclimatisation and re-exposure Journal of Applied Physiology, 116(7).

- Subudhi, A.W., Dimmen, A.C., & Roach, R.C. (2007) Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. Journal of Applied Physiology, 103(1), 177-183.

- Amann, M., Romer, L.M., Subudhi, A.W., Pegelow, D.F., & Dempsey, J.A. (2007). Severity of arterial hypoxemia affects the relative contributions of peripheral vs. central fatigue to exercise performance. Journal of Physiology, 581(1), 389-403.

- Subudhi, A.W., Lorenz, M.C., Fulco, C.S., & Roach, R.C. (2008). Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. American Journal of Physiology - Heart and Circulatory Physiology, 294(1), H164-H171.

- Subudhi, A.W., Miramon, B.R., Granger, M.E., & Roach, R.C. (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. Journal of Applied Physiology, 106(4), 1153-1158.

- Subudhi, A.W., Panerai, R.B, & Roach, R.C. (2009). Acute hypoxia impairs dynamic cerebral autoregulation: Results from two independent techniques. Journal of Applied Physiology, 107(4), 1165-1171.

- Hu, X., Subudhi, A., Xu, P., Asgari, S., Roach, R., & Bergsneider, B. (2009). Inferring cerebrovascular changes from latencies of systemic and intracranial pulses: a model based latency subtraction algorithm. Journal of Cerebral Blood Flow and Metabolism, 29(4), 688-697.

- Subudhi, A.W., Panerai, R.B, & Roach, R.C. (2010). Effects of hypobaric hypoxia on cerebral autoregulation. Stroke**,** published ahead of print 10.1161/STROKEAHA.109.574749.

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- Subudhi, A.W., Olin J.T., Dimmen, A.C., Polaner, D.M., Kayser, B., & Roach, R.C. (2011). Does cerebral oxygen delivery limit incremental exercise performance? Journal of Applied Physiology. 111(6): 1727-1734.

- Asgari, S., Subudhi, A.W., Xu, P., Roach, R.C., Liebeskind, D.S., Bergsneider, B., & Hu, X. (2011). An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. Journal of Neuroscience Methods, 197(1), 171-179.

- Sato, K., Sadamoto, T., Hirasawa, A., Oue, A., Subudhi, A.W., Miyazawa, T., & Ogoh, S. (2012). Differential blood flow responses to CO_2 in human internal and external carotid and vertebral arteries. Journal of Physiology, 590(Pt14): 3277-3290.

- Asgari, S., Gonzalez, N., Subudhi, A.W., Hamilton, R., Vespa, P., Bergsneider, M., Roach, R.C., & Hu, X. (2012). Continuous detection of cerebral vasodilatation and vasoconstriction using intracranial pulse morphological template matching.PLoS One, 7(11):e50795.

- Miyazawa, T., Horiuchi, M., Ichikawa, D., Subudhi, A.W., Sugawara, J., & Ogoh, S. (2012). Face cooling with water mist increases cerebral blood flow during exercise: effect of changes in facial skin blood flow. Frontiers in Physiology, 3:308.

- Ogoh, S., Sato, K., Nakahara, H., Okazaki, K., Subudhi A., & Miyamoto, T. (2013). Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. Experimental Physiology. 98.3: 692-698.

- Amann, M., Goodall, S., Twomey, R., Subudhi, A.W., Lovering, A.T., & Roach, R.C. (2013). AltitudeOmics: On the consequences of high altitude acclimatization for the development of fatigue during locomotor exercise in humans. Journal of Applied Physiology, 115(5): 634-42.

- Subudhi, A.W., Fan, J.L., Evero, O., Bourdillon, N., Kayser, B., Julian, C.G., Lovering, A.T., & Roach, R.C. (in press). AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery**.** Experimental Physiology

D. Research Support

<u>Current Support</u>

2009 – Present	Medical Education and Research Institute of Colorado (MERIC). Interdisciplinary laboratory investigations in biological sciences. Role: Co-Principal Investigator (w/ Jacqueline Berning and Jeffery Broker).
2010 - 2014	Department of Defense - Applied Research and Advanced Technology Development Award. Prediction of Acute Mountain Sickness Using a Blood-Based Test. Role: Co-Investigator (PI: Robert C. Roach).
2011 - 2014	Department of Defense - Defense Medical Research and Development Program Basic Research Award. AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude. Co-Investigator (PI: Robert C. Roach).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Andrew T. Lovering, PhD	POSITION TITLE Associate Professor of Human Physiology
eRA COMMONS USER NAME (credential, e.g., agency login) LOVERING	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Texas Tech University (TTU), Lubbock, TX	BS	05/95	Biology
TTU School of Medicine (TTUSOM), Lubbock, TX	PhD	05/03	Neurophysiology
University of Wisconsin (UW) School of Medicine and Public Health, Madison, WI	Postdoctoral	06/07	Pulmonary Physiology

A. Personal Statement

The goal of the proposed research is to find the best new approach to improving SOF warfighter performance on rapid exposure to high altitude.

The Lovering Lab is well suited to provide critical expertise towards the aims of this project as I have an extensive background in cardiopulmonary physiology, with specific interest in physiological responses to high altitude and hypoxia. Our lab location is also well suited for the recruitment and screening of altitude naïve subjects. In fact, we have previously worked with Dr. Roach to select and screen altitude naïve subjects for the AltitudeOmics 2012 research expedition to Bolivia. Research conducted in my lab related to hypoxia, high altitude and lung disease has been well funded by the DOD, the AHA, and the ALA. Importantly, we are completing work on a recent grant from the Defense Medical Research & Development Program to study the role of intrapulmonary arteriovenous anastomoses and patent foramen ovale with relation to pulmonary gas exchange efficiency and acute mountain sickness. I have also participated in field research expeditions in Nepal and Tibet to study high altitude physiology through collaborations with other leading scientists in the field. These collaborations have resulted in several peer-reviewed publications and ongoing projects. My previous experiences with team-based research projects have taught me the importance of clear communication between project members, constructing achievable research plans, goals, and budgets.

B. Positions and Honors

Positions and Employment

1993 – 1995	Undergraduate Fellow, TTUSOM, Dept of Pharmacology, Lubbock, TX
1995 – 1996	Research Technician II, TTUSOM, Dept of Pharmacology, Lubbock, TX
1996 – 1998	Research Technician III, TTU, Biology Dept, Lubbock, TX
1998 – 2003	Graduate Research Fellow, TTUSOM, Dept of Physiology, Lubbock, TX
2003 – 2007	Postdoctoral Fellow, UW School of Medicine & Public Health, Madison, WI
2007 – 2012	Assistant Professor, University of Oregon, Human Physiology, Eugene, OR
2012 – Present	Associate Professor, University of Oregon, Human Physiology, Eugene, OR

Other Experience and Professional Memberships

1999 – Present	American Physiological Society (APS) Member
2005	APS Minority Travel Fellowship Mentor for Carmen Troncoso
2012	NIGMS Minority Summer Fellow Mentor for Juan Wilkins

Honors

1993 – 1995	ASPET Summer Fellowship for Undergraduate Research
1998 – 2003	U.S. Department of Education GAANN Fellowship
2000	Travel Award, IV World Congress; Mount Med & High Altitude Physiol–Chile
2001	Travel Award, World Federation of Sleep Res. Societies III Conf–Uruguay
2002 – 2003	Achievement Rewards for College Scientists (ARCS) Foundation Scholarship
2003	Sleep Research Society Trainee Research Merit Award – Chicago
2003	The Outstanding Graduate Student, TTUSOM
2003 – 2006	NIH Postdoctoral Fellowship in Respiratory Neurobiology
2005 – 2007	NIH Clinical Loan Repayment Program
2010	Sacred Heart Foundation PeaceHealth Clinical Research Recognition Award
2010	APS Giles F. Filley Memorial Awards for Excellence in Resp Physiol & Med
2012	University of Oregon Faculty Excellence Award

C. Selected Peer-reviewed Publications (Selected from 39 peer-reviewed publications)

Most Relevant to the Current Application

1. Subudhi, A.W., J-L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, **A.T. Lovering**, R. Panerai R.C. Roach. AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness" *J Appl Physiol* 116: 724-729, 2014. PMID: 24371013
2. Fan, J-L, A.W. Subudhi, O. Evero, N. Bourdillon, B. Kayser, **A.T. Lovering**, R.C. Roach. AltitudeOmics: Enhanced cerebrovascular function with high altitude acclimatisation and re-exposure *J Appl Physiol* 116: 911-918, 2014. PMID: 24356520
3. Subudhi, A.W., J-L. Fan, O. Evero, N. Bourdillon, B. Kayser, C.G. Julian, **A.T. Lovering**, R.C. Roach. AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. *Exp Physiol 99 (5) 772-781;2014.* PMID: 24243839
4. Subudhi, A.W., J. Bucher, N.Bourdillon, C.Davis, J.Elliott, M.Eutermoster, O.Evero, J-L. Fan, S.Jameson-Van Houten, C.G. Julian, J.Kark, S.Kark, B.Kayser, J.P. Kern, S. Kim, C. Lathan, S.S. Laurie, **A.T. Lovering**, R. Paterson, D. Polaner, B.J. Ryan, J. Spira, N.B. Wachsmuth, R.C. Roach. AltitudeOmics: The Integrative Physiology of Human Acclimatization to Hypobaric Hypoxia and Its Retention Upon Reascent. *PLOSone* 9(3):e92191, 2014. PMCID: PMC3962396
5. Foster, G.E., P.N. Ainslie, M. Stembridge, T.A. Day, A. Bakker, S.J. Lucas, N.C. Lewis, K.R. Burgess, D. MacLeod, **A.T. Lovering.** Pulmonary hemodynamics and shunting: a comparison of sea-level inhabitants to high altitude Sherpas. *J Physiol* 592(6): 1397-1409, 2014. PMID: 24396057

Additional recent publications of importance to the field (in chronological order)

1. Amann, M., M.W. Eldridge, **A.T. Lovering**, M.K. Stickland, D.F. Pegelow and J.A. Dempsey. Arterial oxygen content influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue. *J Physiol* 575(3):937-952, 2006. PMID:16793898, PMCID: PMC1995675
2. Romer, L.M., M. Amann, H.C. Haverkamp, **A.T. Lovering**, D.F. Pegelow and J.A. Dempsey. Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans *Am J Physiol:Regul Integr Comp Physiol* 292(1):R598-606, 2007. PMID: 16959862
3. **Lovering, A.T.**, L.M. Romer, H.C. Haverkamp, D.F. Pegelow, J.S. Hokanson and M.W. Eldridge. Intrapulmonary shunting and pulmonary gas exchange during normoxic and hypoxic exercise in healthy humans. *J Appl Physiol* 104:1418-1425, 2008. PMID: 18292301
4. Laurie, S.S., X. Yang, J.E. Elliott, K.M. Beasley & **A.T. Lovering**. Hypoxia-induced intrapulmonary arteriovenous shunting at rest in healthy humans *J Appl Physiol* 109:1072-1079, 2010. PMID: 20689088
5. Elliott, J.E., Y. Choi, S.S. Laurie, X. Yang, I.M. Gladstone & **A.T. Lovering.** Gas bubble composition does not affect detection of inducible intrapulmonary arteriovenous shunt at rest or during exercise in normoxia, hypoxia or hyperoxia. *J Appl Physiol* 110: 35-45, 2011. PMID: 20847134
6. **Lovering, A.T.**, M.K. Stickland, M. Amann, J.S. Hokanson and M.W. Eldridge. Effect of a patent foramen ovale on pulmonary gas exchange during normoxic and hypoxic exercise. *J Appl Physiol* 110: 1354–1361, 2011. PMID: 21372097, PMCID: PMC3290103
7. Laurie, S.S., J.E. Elliott, R.D. Goodman, **A.T. Lovering**. Catecholamine-induced intrapulmonary arteriovenous shunting in healthy humans at rest. *J Appl Physiol 113(8): 1213-1222, 2012*. PMID:22858627

8. Amann, M., S. Goodall, R. Twomey, A.W. Subudhi, **A.T. Lovering**, R.C. Roach. AltitudeOmics: On the consequences of high altitude acclimatization for the development of fatigue during locomotor exercise in humans *J Appl Physiol* 115(5): 634-42, 2013*.* PMID:23813531
9. Goodall, S. M., R. Twomey, M. Amann, E.Z. Ross, **A.T. Lovering**, Lee M. Romer, A.W. Subudhi, R.C. Roach. AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude. *Acta Physiol* 210 (4): 875–888, 2014. PMID: 24450855
10. HC Norris, TS Mangum, JW, Duke, TB Straley, RD Goodman, JA Hawn & **A.T. Lovering**. Exercise- and hypoxia-induced blood flow through intrapulmonary arteriovenous anastomoses is reduced in older adults. *J Appl Physiol 2014 doi:10.1152/japplphysiol.01125.2013* ***(In Press)***

D. Research Support

Ongoing Research Support

AHA Scientists Development Grant Lovering (PI) 07/01/2009 – 06/30/2014
GRANT # 2280238
Title: Cardiopulmonary responses to exercise & hypoxia in adult survivors of Bronchopulmonary Dysplasia
Major Goals: To determine the cardiopulmonary responses to exercise and hypoxia stress in full term, preterm and preterm subjects with BPD.
Role: PI

Defense Medical Research & Development Program Lovering (PI) 10/01/2010 – 09/30/2014
GRANT # W81XWH-10-2-0114/#DM1027581 JTCG5 TATRC
Title: Prediction of susceptibility to acute mountain sickness using hypoxia-induced intrapulmonary arteriovenous shunt and intracardiac shunt fractions
Major Goals: Develop a method to predict susceptibility of healthy humans to acute mountain sickness.
Role: PI

AHA Predoctoral Fellowship Elliott (PI) 07/01/2012 – 06/30/2014
Title: Epinephrine-induced recruitment of intrapulmonary arteriovenous shunt in healthy humans at rest
Major Goals: To determine the role of epinephrine in opening intrapulmonary shunts at rest and their role in gas exchange efficiency
Role: Mentor

Evonuk Memorial Graduate Fellowship in Environ Physiol Elliott (PI) 2013 – 2014
Title: Mechanisms of pulmonary gas exchange efficiency: Revisiting the paradigm
Major Goals: Demonstrate that blood flow through IPAVA provides a source of venous admixture that impairs pulmonary gas exchange efficiency.
Role: Mentor

Med. Research Foundation of OR Early Clinical Investigator Duke (PI) 12/01/2013 – 11/30/2014
GRANT # 1348
Title: Mitigation of cardiopulmonary sequelae associated with bronchopulmonary dysplasia
Major Goals: 1) Identify an excessive work of breathing as a limitation to exercise performance in adults with a history of extreme preterm birth (<30 weeks gestational age). 2) Demonstrate that exercise-induced pulmonary capillary hemorrhage occurs in adults with a history of extreme preterm birth (<30 weeks gestational age).
Role: Mentor

Completed Research Support

PeaceHealth Translational Research Award Program Lovering (PI) 07/01/2010 – 06/30/2011
GRANT # 429234
Title: Oxygen mediation of intrapulmonary arteriovenous anastomoses in healthy humans.
Major Goals: quantify shunt fraction under a variety of physiologic conditions in healthy humans.
Role: PI

Principle Investigator (**Lovering, Andrew Thomas**):

American Thoracic Society/American Lung Association Lovering (PI) 09/01/2010 – 08/31/2012
GRANT #C-10-014
Title: Prevention of intrapulmonary arteriovenous shunting in patients with COPD
Major Goals: Determine the role of arterial desaturation in the regulation of intrapulmonary arteriovenous pathways in subjects with COPD
Role: PI

Alberta Health Services Emerging Research Teams Grant Thébaud (PI) 10/01/2009 – 09/30/2013
GRANT # RES0002582
Title: Cardio-respiratory function, school age abilities and quality of life in extremely low birth weight infants.
Major Goals: Determine the long-term cardiopulmonary outcomes of children born prematurely.
Role: Co-I

Evonuk Memorial Graduate Fellowship in Environ Physiol Laurie (PI) 2010 – 2011
Title: Intrapulmonary Arteriovenous Anastomoses Contribute to Pulmonary Gas Exchange Inefficiency during Exercise in Healthy Humans.
Major Goals: Develop a refined nuclear medicine technique to quantify intrapulmonary shunt fraction
Role: Mentor

Defense University Research Instrumentation Program (DURIP) Halliwill (PI) 06/15/2011 – 06/14/2012
Title: Assessment of blood flow and perfusion during challenges to homeostasis in humans
Major Goals: To purchase a transcranial Doppler system and a Near Infrared Spectroscopy System
Role Co-PI

Defense University Research Instrumentation Program (DURIP) Lovering (PI) 06/15/2012 – 06/14/2013
Title: Multidimensional ultrasound assessment of blood flow & perfusion during challenges to homeostasis in humans
Major Goals: To purchase a Philips ie33 3D Doppler Ultrasound System
Role: PI

RESEARCH & RELATED Senior/Key Person Profile

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Robert	Corwine	Roach Jr.	

Position/Title: Associate Professor
Department: 20353 -- SOM-EM MED CLINICAL

Organization Name: University of Colorado Denver
Division:

* Street1: 12469 E 17th PL
Street2:

* City: Aurora
County:
* State: CO: Colorado
Province:

* Country: USA: UNITED STATES
* Zip / Postal Code: 80045

*Phone Number	Fax Number	* E-Mail
303-724-1671		robert.roach@ucdenver.edu

Credential, e.g., agency login: Roach.R

*** Project Role:** PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	Bio_Dr._Robert_Corwine_Roach_Jr._0.pdf	MIMETYPE
Attach Current & Pending Support	otherSupport_Dr._Robert_Corwine_Roach_Jr._0.pdf	MIMETYPE

PROFILE - Senior/Key Person 1

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Andrew	W.	Subudhi	Ph.D.

Position/Title: ERA/INFOED
Department: 60065 -- ADM AVCRC

Organization Name: University of Colorado Denver
Division:

* Street1: 12469 east 17th place
Street2:

* City: aurora
County:
* State: CO: Colorado
Province:

* Country: USA: UNITED STATES
* Zip / Postal Code: 80045

*Phone Number	Fax Number	* E-Mail
303/724-1630		asubudhi@uccs.edu

Credential, e.g., agency login:

*** Project Role:** Co-PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	Bio_Andrew_W._Subudhi_Ph.D._1.pdf	MIMETYPE
Attach Current & Pending Support	otherSupport_Andrew_W._Subudhi_Ph.D._1.pdf	MIMETYPE

PROFILE - Senior/Key Person 2				
Prefix	* First Name	Middle Name	* Last Name	Suffix
	John		Davis	

Position/Title:

Department: Alma College

Organization Name: Alma College

Division:

* Street1: 614 West superior street

Street2:

* City: alma

County:

* State: MI: Michigan

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 48801

*Phone Number	Fax Number	* E-Mail
989-463-7158		davisj@alma.ed

Credential, e.g., agency login:

*** Project Role:** Other (Specify)

Other Project Role Category: Subcontract PI

	File Name	Mime Type
***Attach Biographical Sketch**	Bio_John_Davis_2.pdf	MIMETYPE
Attach Current & Pending Support	otherSupport_John_Davis_2.pdf	MIMETYPE

	File Name	Mime Type
ADDITIONAL SENIOR/KEY PERSON PROFILE(S)		
Additional Biographical Sketch(es) (Senior/Key Person)		
Additional Current and Pending Support(s)		

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel listed on the budget page.

NAME	POSITION TITLE
Robert C. Roach, Ph.D.	Associate Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training).

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
The Evergreen State College, Olympia, WA	B.S.	1979	Biochemistry
Cornell University, Ithaca, NY	M.S.	1985	Nutritional Science
University of New Mexico, Albuquerque, NM	Ph.D.	1994	Exercise Physiology

RESEARCH AND PROFESSIONALEXPERIENCE: Concluding with present position, list in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List in chronological order, the titles, all authors and complete references to all publications during the past 3 years and to representative earlier publication pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

POSITIONS AND EMPLOYMENT

1989-2005 Associate Scientist, Siberian-Alaskan Medical Research Exchange, Section Cold Altitude Physiology, University of Alaska, Anchorage, AK
1990-1994 Research Physiologist, Oxygen Transport Program, The Lovelace Institutes, Albuquerque, NM
1993-2005 Consultant, Life Support Systems, Odyssey Around the World Balloon Flight, Albuquerque, NM
1994-1996 Associate Scientist, Cardiopulmonary Physiology, Institute Basic Applied Medical Research, The Lovelace Institutes, Albuquerque, NM
1996-1998 Alfred Benzon Research Fellow, Copenhagen Muscle Research Center, Copenhagen, Denmark.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

1998-1999 Visiting Professor, Department of Life Sciences, New Mexico Highlands University, Las Vegas, NM
1999-2000 Research Assistant Professor, Department Life Sciences, New Mexico Highlands University, Las Vegas, NM
1999-2003 Clinical Assistant Professor, Department Medicine, University of New Mexico, Albuquerque, NM
1999-2002 Clinical Assistant Professor, Department Surgery, Div Emergency Medicine, UCHSC
1999-present Co-Chairman, International Hypoxia Symposia (www.hypoxia.net)
2001-2003 Scientist, New Mexico Resonance, Albuquerque, NM
2003-2010 Associate Director and Chief, Research Division, Altitude Research Center UCHSC, Denver, CO
2010-present Director, Altitude Research Center, University of Colorado Denver, Denver, CO

PROFESSIONAL MEMBERSHIPS
American Physiological Society; American College of Sports Medicine; American Association for the Advancement of Science; American Alpine Club; International Society for Mountain Medicine

REVIEW AND REFEREE WORK
Appointed, Editorial Board, Journal of Applied Physiology, 2006 to present
Appointed, Editorial Board, High Altitude Medicine and Biology, 2006 to present
Appointed, Editorial Board, Medicine Science Sports and Exercise, 2005 to 2011
Appointed, Section Editor, Hypoxia, Extreme Medicine and Physiology, BMC Journals, 2011-present.
Invited Reviewer, DOD Brain Injury Study Section, American Institute of Biological Science, 2009-2010.

HONORS AND AWARDS
Elected Fellow, American College of Sports Medicine (FACSM), fall 2004.
Appointed, American Physiological Society Porter Scholarship Selection Committee, 2005-2008
Appointed, American College of Sports Medicine, Constitution, Bylaws and Operating Codes Committee, 2006-2009
Appointed, American College of Sports Medicine, Promotions and Fellowship Committee, 2011 to present

ONGOING RESEARCH SUPPORT
DMDRP W81XWH-11-2-0034 (Roach, PI) 12/20/2010-12/20/2015
Prediction of acute mountain sickness using a blood-based test
This project aims at developing a rapid, cost-effective, pre-ascent screening test to predict individual risk of acute mountain sickness (AMS) for military use.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

DMDRP W81XWH-11-2-0040 (Roach, PI) 01/01/2011-6/30/2014
AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude
This project aims at advancing high-altitude medical research by discovering the basic molecular mechanisms of acclimatization that protect soldiers from high altitude illness.

DARPA (PI: Irwin, D, Co-PI: Roach) (01/01/2012-12/31/2014)
Rapid Acclimatization to Hypoxia at Altitude.
The advancement of high-altitude medical research by discovering novel preventive measures for acute mountain sickness.

PUBLICATIONS (from 115 total publications, 19 in last three years)

1. Asgari S, Subudhi AW, Roach RC, Liebeskind DS, Bergsneider M, Hu X. An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. Journal of neuroscience methods 2011;197:171-9.
2. Browne VA, Toledo-Jaldin L, Davila RD, et al. High-end arteriolar resistance limits uterine artery blood flow and restricts fetal growth in preeclampsia and gestational hypertension at high altitude. American journal of physiology Regulatory, integrative and comparative physiology 2011;300: R1221-9.
3. Julian CG, Subudhi AW, Wilson MJ, Dimmen AC, Pecha T, Roach RC. Acute mountain sickness, inflammation, and permeability: new insights from a blood biomarker study. Journal of Applied Physiology 2011;111:392-9.
4. Roach R, Hackett P, Kayser B. Pro: Rebuttal. High Altitude Medicine & Biology 2011;12:27-.
5. Roach R, Kayser B, Hackett P. Pro: Headache should be a required symptom for the diagnosis of acute mountain sickness. High altitude medicine & biology 2011;12:21-2; discussion 9.
6. Subudhi AW, Dimmen AC, Julian CG, Wilson MJ, Panerai RB, Roach RC. Effects of acetazolamide and dexamethasone on cerebral hemodynamics in hypoxia. Journal of applied physiology 2011;110:1219-25.
7. Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B, Roach RC. Does cerebral oxygen delivery limit incremental exercise performance? Journal of applied physiology 2011;111:1727-34.
8. Wilson MJ, Julian CG, Roach RC. Genomic analysis of high altitude adaptation: innovations and implications. Current sports medicine reports 2011;10:59-61.
9. Ezzati M, Horwitz ME, Thomas DS, et al. Altitude, life expectancy and mortality from ischaemic heart disease, stroke, COPD and cancers: national population-based analysis of US counties. J Epidemiol Community Health 2012;66:e17.
10. Olin JT, Dimmen AC, Subudhi AW, Roach RC. A simple method to clamp end-tidal carbon dioxide during rest and exercise. Eur J Appl Physiol 2012;112:3439-44.
11. Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC. AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans. Journal of applied physiology 2013;115:634-42.

12. Fan JL, Subudhi AW, Evero O, et al. AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure. Journal of applied physiology 2014;116:911-8.
13. Goodall S, Twomey R, Amann M, et al. AltitudeOmics: exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude. Acta physiologica 2014;210:875-88.
14. Julian CG, Subudhi AW, Hill RC, et al. Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. Journal of applied physiology 2014;116:937-44.
15. Roach EB, Bleiberg J, Lathan CE, Wolpert L, Tsao JW, Roach RC. AltitudeOmics: Decreased reaction time after high altitude cognitive testing is a sensitive metric of hypoxic impairment. Neuroreport 2014.
16. Roach RC, Wagner PD, Hackett PH. Translation in progress: hypoxia. Journal of applied physiology 2014;116:835-6.
17. Subudhi AW, Bourdillon N, Bucher J, et al. AltitudeOmics: the integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent. PLoS One 2014;9:e92191.
18. Subudhi AW, Fan JL, Evero O, et al. AltitudeOmics: cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. Journal of applied physiology 2014;116:724-9.
19. Subudhi AW, Fan JL, Evero O, et al. AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. Experimental physiology 2014.

Current/Pending Support – Roach, R.

DMDRP W81XWH-11-2-0034 (Roach, PI) 12/20/2010-12/20/2015

Prediction of acute mountain sickness using a blood-based test

0.55 FTE

DARPA (**PI: Irwin, D, Co-PI: Roach**) (01/01/2012-04/30/2015)
Rapid Acclimatization to Hypoxia at Altitude.

0.2 FTE

Recently Support

DMDRP W81XWH-11-2-0040 (Roach, PI) 01/01/2011-7/30/2014
AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude

0.45 FTE

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel listed on the budget page.

NAME	POSITION TITLE
Andrew W. Subudhi	Associate Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training).

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
The Colorado College	B.A.	1992	Mathematics
Colorado State University	M.S.	1996	Exercise Science
University of Utah	Ph.D.	2000	Exercise Physiology
University of Colorado Health Science Center	Post Doc	2003-05	Altitude Physiology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List in chronological order, the titles, all authors and complete references to all publications during the past 3 years and to representative earlier publication pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

POSITIONS AND EMPLOYMENT

1997 - 2005 Research Scientist, The Orthopedic Specialty Hospital (TOSH), Intermountain Health Care, Salt Lake City, UT.
2000 - 2008 Adjunct Assistant Professor, University of Utah, Division of Foods & Nutrition, Salt Lake City, UT.
2001 - 2005 Adjunct Assistant Professor, University of Utah, Dept. of Exercise & Sport Science, Salt Lake City, UT.
2005 - 2011 Assistant Professor, University of Colorado at Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2005 - 2011 Assistant Professor, University of Colorado at Denver, Dept. of Surgery, Denver, CO

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). *PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.*

2011 - Present Associate Professor, University of Colorado Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2011 - Present Associate Professor, University of Colorado Denver/Anschutz Medical Campus, Dept. of Emergency Medicine, Denver, CO.

PROFESSIOAL MEMBERSHIPS
1995 – Present: Member of the American College of Sports Medicine
1996 – Present: Certified Strength and Conditioning Specialist (C.S.C.S.)
2000 – Present: Member of the American Physiological Society

HONORS AND AWARDS
Colorado Graduate Fellowship, Colorado State University, 1995
Phi Kappa Phi Honors Society, Colorado State University, 1996
Phi Kappa Phi Honors Society, University of Utah, 1999
Beta Beta Beta Honors Society, University of Colorado at Colorado Springs, 2006
Alpha Epsilon Delta Honors Society, University of Colorado at Colorado Springs, 2006
LAS Outstanding Teaching Award, University of Colorado at Colorado Springs, 2009
Fellow of the American College of Sports Medicine (FACSM), 2009
Innovations in Teaching with Technology. University of Colorado Colorado Springs, 2012

PUBLICATIONS (from 50 total publications)

1. Hagobian T., Jacobs, K.A., Subudhi, A.W., Fattor, J.A., Rock, P.B., Muza, S.R., Fulco, C.S., Braun, B., Grediagin, A., Mazzeo, R.S., Cymerman, A., & Friedlander, A.L. (2006). Cytokine response at high altitude: effects of exercise and antioxidants at 4,300 m. Medicine and Science in Sports and Exercise, 38(2), 276-285.
2. Subudhi, A.W., Jacobs, K.A., Hagobian T.A., Fattor, J.A., Muza, S.R., Fulco, C.S., Cymerman, A., & Friedlander, A.L. (2006). Changes in ventilatory threshold at high altitude: effect of antioxidants. Medicine and Science in Sports and Exercise, 38(8), 1425-1431.
3. Amann, M., Romer, L.M., Subudhi, A.W., Pegelow, D.F., & Dempsey, J.A. (2007). Severity of arterial hypoxemia affects the relative contributions of peripheral vs. central fatigue to exercise performance. Journal of Physiology, 581(1), 389-403.
4. Subudhi, A.W. & Roach, R.C. (2008). Endurance performance at altitude. Current Sports Medicine Reports 7(1), 6-7.
5. Imray C, Wright A, Subudhi A, & Roach R. (2010). Acute mountain sickness: pathophysiology, prevention, and treatment. Progress in Cardiovascular Diseases, 52(6), 467-484.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

6. Olin, J.T., Dimmen, A.C., Subudhi, A.W., & Roach, R.C. (2011). Cerebral blood flow and oxygenation at maximal exercise: The effect of clamping carbon dioxide. Respiratory Physiology & Neurobiology, 175, 176-180.

7. Asgari, S., Subudhi, A.W., Xu, P., Roach, R.C., Liebeskind, D.S., Bergsneider, B., & Hu, X. (2011). An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. Journal of Neuroscience Methods, 197(1), 171-179.

8. Subudhi, A.W., Dimmen, A.C., Julian, C.G., Wilson, M.J., Panerai, R.B., & Roach, R.C. (2011). Effects of acetazolamide and dexamethasone on cerebral hemodynamics in hypoxia. Journal of Applied Physiology, 110(5): 1219-1225.

9. Julian, C.G., Subudhi, A.W., Wilson, M.J., Dimmen, A.C., Pecha T., & Roach, R.C. (2011). Acute mountain sickness, inflammation and permeability: New insights from a blood biomarker study. Journal of Applied Physiology, 111(2): 392-399.

10. Subudhi, A.W., Olin J.T., Dimmen, A.C., Polaner, D.M., Kayser, B., & Roach, R.C. (2011). Does cerebral oxygen delivery limit incremental exercise performance? Journal of Applied Physiology. 111 (6): 1727-1734.

11. Sato, K., Sadamoto, T., Hirasawa, A., Oue, A., Subudhi, A.W., Miyazawa, T., & Ogoh, S. (2012). Differential blood flow responses to CO2 in human internal and external carotid and vertebral arteries. Journal of Physiology, 590(Pt14): 3277-3290.

12. Olin, J.T., Dimmen, A.C., Subudhi, A.W., & Roach, R.C. (2012). A simple method to clamp end-tidal carbon dioxide during rest and exercise. European Journal of Applied Physiology, 112(9): 3439-3444.

13. Miyazawa, T., Horiuchi, M., Ichikawa, D., Subudhi, A.W., Sugawara, J., & Ogoh, S. (2012). Face cooling with water mist increases cerebral blood flow during exercise: effect of changes in facial skin blood flow. Frontiers in Physiology, 3:308.

14. Asgari, S., Gonzalez, N., Subudhi, A.W., Hamilton, R., Vespa, P., Bergsneider, M., Roach, R.C., & Hu, X. (2012). Continuous detection of cerebral vasodilatation and vasoconstriction using intracranial pulse morphological template matching.PLoS One, 7(11):e50795.

15. Schommer, K., Menold, E., Subudhi, A.W., Bartsch, P. (2012). Health risks for athletes at moderate altitude and normobaric hypoxia. British Journal of Sports Medicine, 46(11): 828-32.

16. Lundby, C., Millet, G.P., Calbet, J.A., Bartsch, P., & Subudhi, A.W. (2012). Does 'altitude training' increase exercise performance in elite athletes? British Journal of Sports Medicine, 46(11): 792-795.

17. Bergeron, M.F., Bahr, R., Bartsch, P., Bourdon, L., Calbet, J.A., Carlsen, K.H., Castagna, O., Gonzalez-Alonso, J., Lundby, C., Maughan, R.J., Millet, G., Mountjoy, M., Racinais, S., Rasmussen, P., Singh, D.G., Subudhi, A.W., Young, A.J., Soligard, T., & Engebretsen, L. (2012). International Olympic Committee consensus statement on thermoregulatory and altitude challenges for high-level athletes. British Journal of Sports Medicine, 46(11): 770-779.

18. Ogoh, S., Sato, K., Nakahara, H., Okazaki, K., Subudhi A., & Miyamoto, T. (2013). Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. Experimental Physiology. 98.3: 692-698.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

19. Amann, M., Goodall, S., Twomey, R., Subudhi, A.W., Lovering, A.T., & Roach, R.C. (2013). AltitudeOmics: On the consequences of high altitude acclimatization for the development of fatigue during locomotor exercise in humans. Journal of Applied Physiology. 115(5): 634-642.

20. Goodall S., Twomey R., Amann M., Ross E.Z., Lovering A.T., Romer L.M., Subudhi A.W., Roach R.C. (2014). AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatisation to high altitude. Atca Physiologica (Oxford), 210(4):875-88.

21. Julian, C.G., Subudhi, A.W., Hill, R.C., Wilson, M.J., Dimmen, A.C., Hansen, K.C., & Roach, R.C. (2014). Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness and in humans. Journal of Applied Physiology, 116(7):937-44.

22. Fan, J.L., Subudhi, A.W., Evero, O., Bourdillon, N., Kayser, B., Lovering, A.T., & Roach, R.C. (2014). AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high altitude acclimatisation and re-exposure. Journal of Applied Physiology, 116(7):911-8.

23. Subudhi, A.W., Fan, J.L., Evero, O., Bourdillon, N., Kayser, B., Julian, C.G., Lovering, A.T., Panerai, R.B., & Roach, R.C. (2014). AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. Journal of Applied Physiology, 116(7):724-9.

24. Subudhi AW, Bourdillon N, Bucher J, Davis C, Elliott JE, Eutermoster M, Evero O, Fan JL, Jameson-Van Houten S, Julian CG, Kark J, Kark S, Kayser B, Kern JP, Kim SE, Lathan C, Laurie SS, Lovering AT, Paterson R, Polaner D, Ryan BJ, Spira J, Tsao JW, Wachsmuth NB, Roach RC (2014). AltitudeOmics: the integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent. PLoS One; 9(3):e92191

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27. Ainslie, P.N. & Subudhi, A.W. (2014). Cerebral blood flow at high altitude. High Altitude Medicine and Biology, 15(2):133-40.

Current/Pending Support – Subudhi, A.

DMDRP W81XWH-11-2-0034 (Roach, PI) 12/20/2010-12/20/2015

Prediction of acute mountain sickness using a blood-based test

0.15 FTE

DARPA (**PI: Irwin, D, Co-PI: Roach**) (01/01/2012-04/30/2015)
Rapid Acclimatization to Hypoxia at Altitude.

0.1 FTE

Recently Support

DMDRP W81XWH-11-2-0040 (Roach, PI) 01/01/2011-7/30/2014
AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude

0.25 FTE

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel listed on the budget page.

NAME	POSITION TITLE
John E. Davis	Charles A.Dana Professor of Integrative Physiology

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training).

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Kenyon College, Gambier, Ohio.	Bachelor of Arts	1971-1975	Biology
State University College at Buffalo, Buffalo, New York.	Master of Science	1976-1978	Biology
State University of New York at Buffalo, Buffalo, New York.	Doctor of Philosophy	1981-1984	Exercise Science
The Johns Hopkins University, Baltimore, Maryland.	Post-Doctoral Fellow	1984-1985	Environmental Physiology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List in chronological order, the titles, all authors and complete references to all publications during the past 3 years and to representative earlier publication pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

Professional Experience:

1985 - 1991	Assistant Professor, Department of Exercise and Health Science, Alma College, Alma, Michigan.
1991-1997	Associate Professor and Department Chair, Department of Exercise and Health Science, Alma College, Alma, Michigan.
1999-2000	Visiting Professor, Department of Bioscience, University of Hertfordshire, Hatfield, United Kingdom
1997-2003	Professor and Department Chair, Department of Exercise and Health Science, Alma College, Alma, Michigan.
2013-2104	Visiting Professor, Department of Movement Sciences, Utah State Unviersity, Logan, Utah
2003-present	Charles A. Dana Professor of Integrative Physiology and Health Science. Alma College, Alma, Michigan

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). *PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.*

Honors:

Victor M. Hawthorne New Investigator Award- 1988
Alma College Barlow Award for Faculty Excellence - 1990, 1997
Nominated by Alma College for Carnegie Foundation Professor of the year award - 1996, 1997,1999
Posey Award for Research and Teaching - 2000
Awarded Charles A. Dana Endowed Professorship - 2003

Advisory Committees:

National Science Foundation STEP(Science Talent Expansion Program) Advisory Committee 2012 - 2014

Relevant publications including published abstracts:

Fortney, S.M., K.H. Hyatt, J.E. Davis, and J.M. Vogel. Changes in Body Fluid Compartments During a 28-Day Bed Rest. Aerospace Medicine: 62: 97-104, 1991.

Frey, M.A., C. Lathers, J. E. Davis, S. Fortney, and J.B. Charles. Cardiovascular responses to standing: Effect of hydration. J. Clin. Pharmacol. 34:387-393, 1994.

Davis, J.E., W.R. Pourcho, and C.M. Thomas. Effect of maximal exercise on autonomic control of heart rate during orthostatic stress. In Environmental Ergonomics:Recent Progress and New Frontiers Editors: Y. Shapiro, D.S. Moran, and Y. Epstein. Freund Pub. House. London, 1996. p. 381-385.

Davis, J.E. and S.M. Fortney. Effect of Fluid Ingestion on Orthostatic Responses Following Acute Exercise. International Journal of Sports Medicine 18(3):174-178, 1997.

Davis, J.E., K.E. Horwood, and G.K. DeJong. Effects of Exercise During Simulated Microgravity on Postural Control. Aviation, Space, and Environmental Medicine 68:392-395, 1997.

Ventline, H.L., L.M. Girodano, M.J. Luetkemeier, and J.E. Davis. Effects of Moderate Altitude on Peripheral Sweating. Medicine and Science and Sports and Exercise 36(5): S109, 2004.

Hawkins, K., B. hauser, J.E. Davis, and M.J. Luetkemeier. Effect of Altitude on Lactate Removal Rates Following High-Intensity Exercise. Medicine and Science and Sports and Exercise 36(5): S109, 2004.

Montoye, A. , C. McCue, N. Garvin, B. Gervasi, and J. E. Davis.Effect of Moderate Altitude on EPOC following Maximal Exercise. Medicine and Science and Sports and Exercise 41(5): S228, 2009.

L. J. Hasler, D. Johnson, M. J. Luetkemeier, A. Carlson, Katy J. Green, and J. E. Davis. Effects of exercise at moderate altitude on sweat composition and sweat rates. Medicine and Science and Sports and Exercise 41(5): S371, 2009.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). ***PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.***

Davis, J.E., S. A. Swanton, G.L. Gaskell, and K. Walsh. Effect of Moderate Altitude on Lactate Clearance During Active and Passive Recovery. Medicine and Science and Sports and Exercise 41(5): S568, 2009.

Hrutkay, S., J. Thorington, S. Lux and J.E. Davis. Effects of Acute and Chronic Exposure to Altitude on Forearm Blood Flow and Reactive Hypermia. Medicine Science and Sports in Exercise 43(5): S446, 2011.

Grant, A, M. Perez, and J.E. Davis. Effect of Altitude of Residence on the Cardiovascular Responses to Dynamic and Isometric Hand-Grip. Medicine Science and Sports in Exercise 44(5): S667, 2012.

Gooding, B., J. Cole, , D. Hicks, M. Clark, and J.E. Davis. Effects of Moderate Altitude on Oxygen Debt, Oxygen Deficit, and the Onset of Blood Lactate during Exercise. Medicine Science and Sports in Exercise 45(5): S351, 2013.

Jongerkrijg, L., S. Clancy, J. K. Murawske, and J. E. Davis. Effects of Aerobic and Anaerobic Training on Aerobic Capacity and Blood Hematology at 3400 meters. Medicine Science and Sports in Exercise 45(5): S486, 2013.

Davis , J.E., D.R. Wagner, J. Thorington,and C. Schall. Orthostatic Responses at 4860 m in Low, Moderate, and High Altitude Residents. High Altitude Medicine and Biology 14(3): 251-254, 2013.

Wagner, D.R., J. E Davis, T. Payne, and W. Hussain. Muscle Oxygenation during Dynamic and Isometric Exercise in High Altitude-Resident Guides, Climbers and Tourists at 4810 m. High Altitude Medicine and Biology 15 (2): A-273, 2014.

Current and Pending Support
Investigator: John Davis

Support: Current
Role on Project: Principal Investigator

Project/Proposal Title: PRISM: Positive Routes Into Science and Mathematics

Source of Support: National Science Foundation – Science Talent Expansion Program (STEP)

Total Award Amount:
Total Award Period Covered: 09/15/09- 09/01/15

Location of Project: Alma College

Support: Current
Role on Project: Principal Investigator

Project/Proposal Title: e-STEM: Enhancing STEM Education and Practice

Source of Support: Herbert H. and Grace A. Dow Foundation

Total Award Amount:
Total Award Period Covered: 07/15/14- 09/01/16

Location of Project: Alma College

Support: Pending
Role on Project: Co-Principal Investigator

Project/Proposal Title:
e-PRISM: Changing the Way We Teach Undergraduate Science Students using Inquiry-Based Strategies and Undergraduate Research

Source of Support: National Science Foundation – Improving Undergraduate Science Education (IUSE)

Total Award Amount:
Total Award Period Covered: 08/15/14- 08/01/16

Location of Project: Alma College

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved?	● **Yes**	❍ **No**	
1.a. If YES to Human Subjects			
Is the IRB review Pending?	● **Yes**	❍ **No**	
IRB Approval Date:			
Exemption Number:	__ 1 __ 2 __ 3 __ 4 __ 5 __ 6		
Human Subject Assurance Number	00005070		
2. * Are Vertebrate Animals Used?	❍ **Yes**	● **No**	
2.a. If YES to Vertebrate Animals			
Is the IACUC review Pending?	❍ **Yes**	❍ **No**	
IACUC Approval Date:			
Animal Welfare Assurance Number			
3. * Is proprietary/privileged information included in the application?	❍ **Yes**	● **No**	
4.a. * Does this project have an actual or potential impact on the environment?	❍ **Yes**	● **No**	
4.b. If yes, please explain:			
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?	❍ **Yes**	❍ **No**	
4.d. If yes, please explain:			
5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators?	❍ **Yes**	● **No**	
5.b. If yes, identify countries:			
5.c. Optional Explanation:			
6. * Project Summary/Abstract	Project_Abstract		Mime Type: application/pdf
7. * Project Narrative	Project_Narrative		Mime Type: application/pdf
8. Bibliography & References Cited	Bibliography_final		Mime Type: application/pdf
9. Facilities & Other Resources	Facilities_and_other_Resources		Mime Type: application/pdf
10. Equipment	Equipment		Mime Type: application/pdf
11. Other Attachments	Other_Attachments		Mime Type: application/pdf

PROPOSAL ABSTRACT

Proposal Title: *(120 characters maximum)*

Three New Ideas to Protect Special Forces from the Stress of High Altitude

Keywords: *(6-8 words)*

AMS, HAPE, HACE, hypoxia, nifedipine, methazolamide, quercetin, metformin, nutraceutical

Abstract: (Approximately 250 words)

Background

No major advances have been made in preventing high-altitude illnesses or optimizing performance in the last 25 years. We have identified three compounds, quercetin, nifedipine + methazolamide, and metformin, that we believe will both prevent acute mountain sickness (AMS) and improve performance of special operations forces (SOF) at high altitude based on a series of Department of Defense funded projects aimed at describing the molecular and genetic pathways responsible for successful acclimatization to high altitude.

Hypotheses

We will test the hypotheses that quercetin, nifedipine + methazolamide, and/or metformin will reduce the incidence and severity of AMS and improve physical and cognitive function over three days at altitudes between 10,000 and 13,000 ft.

Study Design

60 males who meet health and physical fitness standards required of SOF will be recruited from sea level. Using a double blind, placebo controlled, matched cohort design, we will evaluate the efficacy of each treatment during a 3-day field study designed to simulate a rapid deployment to high altitude (10,000–13,000 feet). Key measurements will include AMS symptoms, the Army Physical Fitness Test, 3-mile uphill hike with rucksack, and computerized cognitive function assessments.

Relevance

The debilitating effects of high altitude pose a significant threat to SOF. The incurred costs and risks of high-altitude illnesses can be substantial. This proposal directly addresses the need to reduce the risks and improve the performance of SOF missions at high altitude. Each compound proposed can be tested today, and if successful, could be used by SOF tomorrow.

NOTHING ON THIS PAGE IS PROPRIETARY INFORMATION

Facilities and other Resources

The Altitude Research Center (ARC) is situated on the Anschutz Medical Campus (AMC) of the University of Colorado Denver (UCD), in Aurora, Colorado. The ARC laboratory facility, a 5,000 square foot center is dedicated to integrative physiology research in humans.

The ARC is a research facility equipped with specialized altitude related research equipment. The ARC is configured of eight offices, a conference room, a break room, an examination room, and two laboratory spaces. One of the two lab spaces (chamber room) includes a hypobaric chamber facility capable of hosting up to eight research subjects and investigators at altitudes up to 120,000 feet for 12-24 hours. The second laboratory space is reserved for experiments performed at Denver’s altitude. This laboratory space is capable of a wide range of research testing, including but not limited to muscle strength and endurance experiments, as well as biomechanical analysis and energy expenditure.

The general laboratory space in Research Complex 1 (RC1) is approximately 300 sq. ft. and houses two laboratory benches with electricity/water hookups, multiple sinks, and vacuum pumps to conduct wet research. The workbenches are equipped with the following minor equipment: Hettiche Centrifuge, International Clinical Centrifuge (with general-purpose transformer), New Brunswick Scientific G24 Environmental Incubator Shaker, Vortex Genie II, as well as multiple pipettes. In addition to the two lab benches, we have access to a fume hood in that laboratory space. This laboratory space will be utilized to run assay on collected Leadville data. The hallway area of RC 2 is equipped with dedicated electrical outlets supplying Dr. Roach’s -80 lockable storage freezer with power, as well as emergency power in case of an electrical blackout. This system is equipped to send out notifications during freezer distress, such as loss of cooling power, electrical power or other potential hazards that could devastate stored research specimens.

Offices: Offices are located to the sides of the main hallway of the ARC, and range from 233 sq. feet to 115 sq. feet. All offices are equipped with an individual telephone line, internet access through the Universities hard-wired high-speed access. In addition a wireless router allows for Internet access through the Universities wireless network. All offices contain computers, desks, chairs and filing cabinets for their occupants. The conference room is 280 sq. feet and is equipped with a large oval table, 14 chairs, and a storage cabinet. A large dry-erase whiteboard allows for drawing up ideas and agenda’s. A projector, capable of supporting Mac and PC laptop computers, is capable to project presentations during weekly research meetings.

Equipment

Core Equipment includes:

1. Oxymon MkIII near infrared spectrometer for tissue oxygenation measurements.
2. Oxymon MkII near infrared spectrometer for tissue oxygenation measurements.
3. Spencer ST3 transcranial Doppler for measurement of cerebral blood flow velocity.
4. DWL Transcranial Doppler for measurement of cerebral blood flow velocity.
5. Sonosite Micromaxx diagnostic ultrasound for monitoring vascular blood flow and cardiac output.
6. Nexfin HD finger plethysmograph for beat-by-beat blood pressure and cardiac output measurements.
7. Colin 7000 tonometer for beat-by-beat blood pressure monitoring (x2)
8. Respiract respiratory gas mixer for controlling end-tidal concentrations of oxygen and carbon dioxide.
9. Ametek oxygen (S-3a/II) and carbon dioxide (CD-3A) analyzers for metabolic measurements (x2).
10. Oxigraf O2cap oxygen and carbon dioxide analyzers for metabolic measurements (x2).
11. Powerlab 16SP and 16/30 data acquisition systems.
12. Radiometer OSM-3 hemoximeter for hemoglobin and hematocrit measurements (x2).
13. Laboratory Instruments blood gas analyzer.
14. Velotron Elite cycle ergometer for time trial exercise testing.

The Chamber Room, with separate Vacuum Pump Room, connects to the main laboratory. This room houses the 12ft x 28ft modern hypobaric chamber capable of hosting 2-8 research subjects and investigators at altitudes up to 25,000 feet for 12-24 hours.

Minor equipment in the main ARC laboratory to be used for this study and transported to the two data collection sites includes: Nellcor N-595 (measures oxyhemoglobin saturation in the peripheral circulation (2 each)), Criticare 503 (measures oxyhemoglobin saturation in the peripheral circulation (2 each)), Universal Ventilation Meter (measures ventilation via spirometry), O_2Cap Oxygen Analyzer (measures oxygen and carbon dioxide concentrations (2 each)), Powerlab 16/30P and Power lab 16SP (both able to integrate up to 16 analog inputs into a single, time-aligned data file and allowing for real-time and offline manipulation of this data), Ametek O_2 and CO_2 analyzers (measures oxygen and carbon dioxide concentrations (2 each)), Vacuumed Metabolic Measurement System (measures ventilation, respiratory gases, and oxygen consumption (2 each)), SECA portable scale (weight measurement of research participants during study), as well as a Samaritan SED defibrillator pad (for basic life support). In addition we will set up a Sorvall RT 6000 Refrigerated Centrifuge (allows for the separation of 15-50 mL tubes at speeds of up to 6,000 revolutions per minute), the Jouan BR 3.11 Centrifuge (separates 5-50mL tubes), and the LW Scientific Microhematocrit Centrifuge (spins down twenty-four 75mL capillary tubes).

Bibliography

1. **Alexander SP**. Flavonoids as antagonists at A1 adenosine receptors. *Phytother Res* 20: 1009-1012, 2006.
2. **Amann M, Subudhi AW, and Foster C**. Predictive validity of ventilatory and lactate thresholds for cycling time trial performance. *Scand J Med Sci Sports* 16: 27-34, 2006.
3. **Antezana AM, Antezana G, Aparicio O, Noriega I, Velarde FL, and Richalet JP**. Pulmonary hypertension in high-altitude chronic hypoxia: response to nifedipine. *Eur Respir J* 12: 1181-1185, 1998.
4. **Bartsch P, Bailey DM, Berger MM, Knauth M, and Baumgartner RW**. Acute mountain sickness: controversies and advances. *High Alt Med Biol* 5: 110-124, 2004.
5. **Bartsch P, Maggiorini M, Ritter M, Noti C, Vock P, and Oelz O**. Prevention of high-altitude pulmonary edema by nifedipine. *N Engl J Med* 325: 1284-1289, 1991.
6. **Bogachus LD, and Turcotte LP**. Genetic downregulation of AMPK-alpha isoforms uncovers the mechanism by which metformin decreases FA uptake and oxidation in skeletal muscle cells. *Am J Physiol Cell Physiol* 299: C1549-1561, 2010.
7. **Braun B, Eze P, Stephens BR, Hagobian TA, Sharoff CG, Chipkin SR, and Goldstein B**. Impact of metformin on peak aerobic capacity. *Appl Physiol Nutr Metab* 33: 61-67, 2008.
8. **Cymerman A, Maher JT, Cruz JC, Reeves JT, Denniston JC, and Grover RF**. Increased 2,3-diphosphoglycerate during normocapnic hypobaric hypoxia. *Aviat Space Environ Med* 47: 1069-1072, 1976.
9. **Dajas F, Andres AC, Florencia A, Carolina E, and Felicia RM**. Neuroprotective actions of flavones and flavonols: mechanisms and relationship to flavonoid structural features. *Cent Nerv Syst Agents Med Chem* 13: 30-35, 2013.
10. **Davis JM, Murphy EA, Carmichael MD, and Davis B**. Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *Am J Physiol Regul Integr Comp Physiol* 296: R1071-1077, 2009.
11. **Dean AG, Yip R, and Hoffmann RE**. High incidence of mild acute mountain sickness in conference attendees at 10 000 foot altitude. *J Wilderness Med* 1: 86-92, 1990.
12. **Derman WE, Sims R, and Noakes TD**. The effects of antihypertensive medications on the physiological response to maximal exercise testing. *J Cardiovasc Pharmacol* 19 Suppl 5: S122-127, 1992.
13. **Dudley JA, Weir RK, Yan TC, Grabowska EM, Grimme AJ, Amini S, Stephens DN, Hunt SP, and Stanford SC**. Antagonism of L-type Ca(v) channels with nifedipine differentially affects performance of wildtype and NK1R-/- mice in the 5-choice serial reaction-time task. *Neuropharmacology* 64: 329-336, 2013.
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Project Narrative

Statement of Work

This project will test the efficacy of three compounds we believe will prevent acute mountain sickness (AMS) and optimize performance of special operations forces (SOF) at operationally relevant altitudes of 10,000 to 13,000 feet. To achieve this objective we will recruit 100 altitude-naïve males from near sea level (Alma, Michigan) to select 60 men who meet or exceed basic physical fitness requirements for SOF. These subjects will be assigned to one of four treatment groups (placebo, quercetin, nifedipine + methazolamide, or metformin) and transported to high altitude (Breckenridge, Colorado) to test the efficacy of the treatments on symptoms of acute mountain sickness (AMS) and physical and cognitive performance over a three-day period. Several critical tasks must be accomplished to complete this project. These tasks have been grouped into action items by year, as described below.

Year 1

1. Obtain University of Colorado and Alma College Institutional Review Board (IRB) approval for study.
2. Obtain Department of Defense (DoD) Human Research Protection Office (HRPO) approval for study.
3. Begin recruiting 100 sea level volunteers and screening for inclusion/exclusion criteria.

Year 2

4. Continue recruiting sea level volunteers and screening for inclusion/exclusion criteria
5. Identity top 60 volunteers according to physical fitness test scores
6. Conduct repeated cognitive function and physical fitness testing on 60 volunteers to ensure performance stability.
7. Establish Colorado basecamp in Breckenridge, Colorado.
8. Conduct practice test weekend with sea level staff in Colorado to standardize pertinent procedures.
9. Schedule volunteers for weekend trips to Colorado.
10. Conduct serial weekend testing with 10-20 subjects per group.
11. Conduct daily data entry of AMS, physical performance, and cognitive function scores.
12. Break drug code and analyze final data set to determine effects on AMS, physical performance and cognitive function at high altitude.

Body of Proposal

1. Background

This proposal directly addresses the Broad Agency Announcement for Extramural Biomedical Research and Development W81XWH-USSOCOM-BAA 14-1, Research Areas of Interest *3. Force Health Protection and Environmental Medicine: a. Optimal Acclimatization Strategy; and b. High Altitude Pulmonary Edema/High Altitude Cerebral Edema*.

High altitude illnesses pose a significant threat to special operations forces (SOF) exposed to high altitudes.(20) Unfortunately, no major advances have been made in preventing high-altitude illnesses or optimizing performance in the last 25 years. This lack of progress has had a direct, detrimental effect on all US forces deployed to high altitudes, but is a particular concern to SOF who are asked to perform extremely demanding tasks immediately upon arrival. To remedy this problem, we propose to test three new ideas to rapidly advance SOF performance at high altitude (Figure 1). We understand USSOCOM is interested in immediate solutions. **Each new idea we propose can be tested today, and if successful, could be used by SOF tomorrow.**

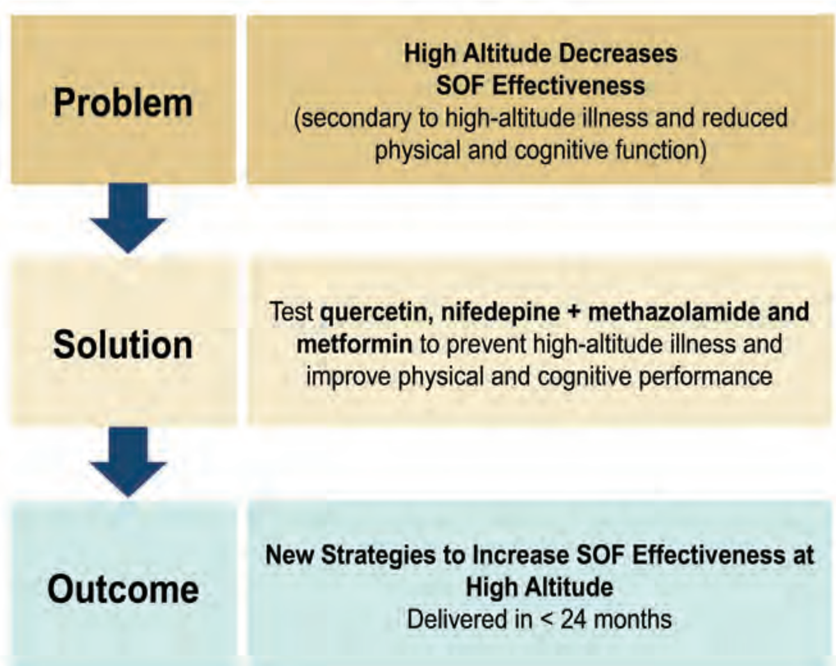


Figure 1. *Graphic description of the overall project to improve SOF effectiveness at high altitude.*

USSOCOM understands the importance of protecting SOF from the challenges of high-altitude illness, so the rationale for these experiments is brief. Acute mountain sickness (AMS) can cause debilitating symptoms, including headache, nausea/vomiting, and fatigue. High-altitude pulmonary edema (HAPE) and high-altitude cerebral edema (HACE) can kill.(20) Although HAPE/HACE incidence is low, their impact can be great. For example, if an operator develops HAPE or HACE, immediate evacuation may be necessary. Such a diversion raises potential catastrophic risks for other troops. The incurred costs and risks of high-altitude illnesses can be substantial. For example, during Operation Enduring Freedom in Afghanistan, ~12% of medevacs and hospital admissions were due to severe AMS/HAPE/HACE.(43) Even in the absence of illness, healthy SOF will experience substantial impairments in physical and cognitive performance at high altitude that may compromise missions. An operator at 14,000 feet may take twice as long to cover the same amount of ground as at sea level. Impaired reaction time and judgment at high altitude may further compromise the health and safety of SOF. **This proposal directly addresses the need to reduce the risks and improve SOF success at high altitude.**

While a two-to-three week period of acclimatization offers near complete protection from high altitude illnesses and substantially improves physical and cognitive function,(53) this strategy is clearly imprac-

tical for SOF who must be ready to deploy at a moment's notice. Quick-acting pharmacological countermeasures to offset the negative effects of high altitude are thus attractive to ensure optimal health and performance at high altitude. Unfortunately, current pharmacological strategies to prevent high-altitude illnesses only work moderately well, or are fraught with side effects and risks.(20, 24) Futhermore, none of the available options offset the decrements in physical and cognitive function inherent upon rapid ascent to high altitude. Our team has recently conducted a series of Department of Defense (DoD) funded studies aimed at understanding the molecular and genetic basis of human adaptation to high altitude as a means to identify novel ways to induce acclimatization and prevent high-altitude illnesses. Based on our research and recent advancements in the field, we have identified three compounds that we believe will improve both health and performance of SOF at high altitude: **quercetin, nifedipine + methazolamide, and metformin.** Quercetin is an over-the-counter nutraceutical that has recently been shown to prevent HACE in animal studies and improve stamina in human exercise trials. Nifedipine and methazolamide, two drugs already approved for use in humans, are effective for preventing HAPE/HACE and may improve physical and cognitive performance at high altitude. Metformin, a drug commonly used to treat diabetes, induces biochemical changes recently linked to successful acclimatization and protection from high-altitude illnesses. **We propose one or more of these approaches will protect SOF from high-altitude illness and substantially improve performance at high altitude.**

What is quercetin and why might it work to improve health and performance at high altitude?

Quercetin is an antioxidant and anti-inflammatory agent widely present in fruits and vegetables.(9) It has been reported to possess antioxidant effects as a free radical scavenger, hydrogen-donating compound, singlet oxygen quencher, and metal ion chelator. Quercetin can also reduce inflammation by scavenging free radicals and attenuating redox-sensitive transcription factors responsible for initiating the inflammatory cascade believed to be involved in the pathophysiology of high-altitude illnesses.(4) **We think quercetin will be effective for preventing high-altitude illnesses because animal studies have shown it to be very effective in preventing cerebral edema.(40, 41, 46)** In three recent studies from independent laboratories, quercetin has been shown to reduce free radical damage in brains of rats exposed to hypoxia. By reducing the amount of free radical damage, inflammatory processes were suppressed, the integrity of cells (e.g. neurons and glia) was preserved, and the severity of cerebral edema was substantially reduced. Figure 2, from Patir and colleagues(41), clearly shows reductions in brain water content in rats exposed to hypoxia (H) when treated with various dosages of quercetin. Note that some quercetin treatments in hypoxia (H+25mg, H+50mg) completely protected against accumulation of brain water, relative to the normoxic control condition (N).

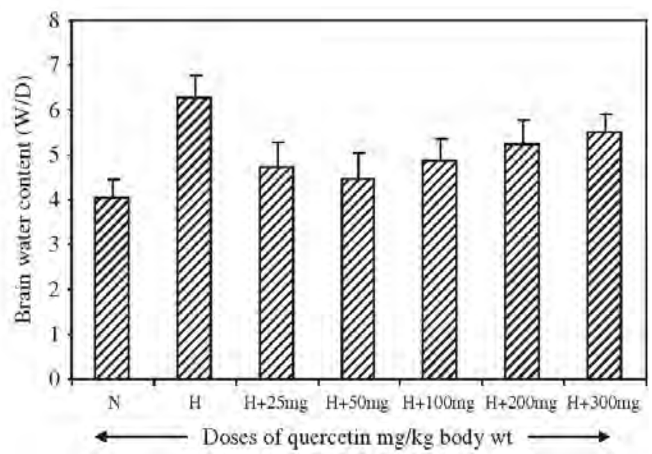


Figure 2. *Quercetin protects against the accumulation of brain water in rats exposed to hypoxia (H), yielding values more similar to those in observed in normoxia (N).*

Impressively, quercetin was also shown to be a more potent anti-inflammatory agent and conferred better protection against cerebral edema than dexamethasone-a powerful anti-inflammatory steroid, that is currently the most effective drug known for preventing and treating AMS, HAPE and HACE.

(20, 24, 32) These intriguing results in animals are directly in line with our recent work in humans suggesting that the anti-inflammatory effects of dexamethasone may explain its ability to prevent AMS.(26) Because quercetin is an over-the-counter nutraceutical with no known side effects,(18, 52) it offers significant advantages over dexamethasone, a prescription-only steroid with a troublesome safety and side-effect profile. If the effects of quercetin in humans are comparable to those in animals, quercetin would likely become the preferred substance to prevent high-altitude illness.

Quercetin has also been shown to improve exercise performance in humans. Two recent meta-analyses concluded that quercetin supplementation (~1000 mg/day) improved endurance running and cycling performance up to 2%.(28, 42) These are remarkable findings since a 2% improvement in performance equates to ~18 seconds in 2-mile run time and could mean the difference between life and death in combat. The mechanism of action is currently unknown, but may be related to quercetin's antioxidant properties,(40) ability to stimulate mitochondrial biogenesis,(10) and/or psychostimulant effects.(1) Based on these ideas, we believe the effect of quercetin on physical performance may be even greater at high altitude for three reasons. First, we have previously shown that antioxidant supplementation at high altitude increases ventilatory threshold, a parameter predictive of running and cycling time-trial performance.(2, 54) Second, successful acclimatization to high altitude is closely associated with improved mitochondrial efficiency.(25) And third, similar psychostimulants (adenosine A1 antagonists), like caffeine, have been shown to improve endurance at high altitude.(16)

Additionally, quercetin appears to improve cognitive function. Data from animal studies suggest that quercetin may protect against memory and cognitive loss.(36, 51, 57) Although there are no documented effects of quercetin on cognitive function in humans, rats treated with quercetin have learned to accomplish maze tasks faster and retain memory better than those receiving a placebo during prolonged periods of hypoxia.(46) The mechanism of action appears to be through antioxidant protection against free radical damage.(51) **Given the documented effects of quercetin for preventing cerebral edema, increasing physical stamina, and preventing cognitive impairments, we believe quercetin will be an effective aid to reduce high-altitude illness and improve performance at high altitude.**

What is nifedipine + methazolamide, and why might it work to improve health and performance at high altitude?

Nifedipine has been shown to prevent and treat HAPE by lowering pulmonary artery pressure and reducing leakage of water from the blood into the lungs.(3, 21, 39) It acts as a calcium channel blocker, but has little effect on cardiovascular function or maximal exercise performance(47) and thus has been recommended as a preferred treatment for hypertension in healthy, active individuals.(12) Although effects of nifedipine on exercise performance at high altitude have not been studied, other studies of pulmonary vasodilators (e.g. sildenafil) have shown improved exercise capacity in hypoxia.(14, 17, 23) Additionally, we believe nifedipine may offset reductions in cognitive performance at high altitude based on recent positive results in animals.(13, 56)

Methazolamide is a cousin of the widely used and successful AMS/HAPE/HACE preventing drug acetazolamide (Diamox).(15, 31, 59) Both methazolamide and acetazolamide block the enzyme carbonic anhydrase and effectively reduce the pH of the brain stem. This in turn stimulates ventilation, improves oxygen transport, and reduces symptoms of high-altitude illnesses.(29, 55) The advantages of methazolamide are that lower doses with fewer side effects seem to achieve equivalent protective effects in animal and human studies.(15, 59) The effects of methazolamide on exercise or cognitive function at high altitude have not been investigated.

Why consider using these two drugs in combination? Nifedipine and methazolamide were independently identified as effective prophylactic treatments for AMS in a recent animal study conducted by our colleague Dr. David Irwin.(31) Unpublished data from Dr. Irwin's lab shows that the combination of nifedipine + methazolamide was almost as effective as dexamethasone in preventing HAPE and HACE in a rat model (Figure 3). This combination of two FDA approved drugs has never been tested in humans. **Based on the animal studies by Dr. Irwin and the effectiveness of each drug alone in humans, we believe the combination of nifedipine + methazolamide will be an effective aid to reduce high-altitude illness and improve performance at high altitude.**

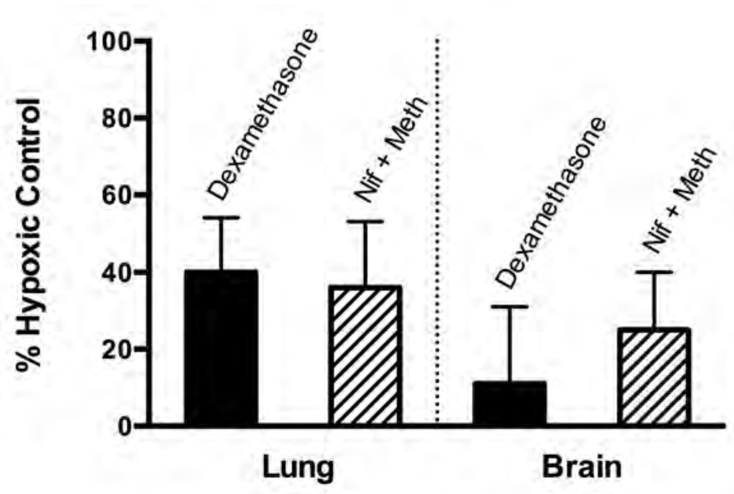


Figure 3. *Dexamethasone was the best drug of 1000's tested in reducing both brain and lung fluid leak compared to control ratsduring high altitude exposure. The next best was nidefedipine and methazolamide, the combination that will be tested in this proposal.*

What is metformin, and why might it work to improve health and performance at high altitude?
In a recent DoD-funded study, we sought to isolate molecular mechanisms responsible for human acclimatization to high altitude with the ultimate goal of identifying pharmacological strategies to help stimulate acclimatization before troops are deployed to high altitude. Since acclimatized humans are nearly completely protected from high-altitude illness and have better physical and cognitive function than they do upon exposure to high altitude, we reasoned that any factor responsible for initiating acclimatization could potentially be responsible for the positive benefits achieved with full acclimatization. Based on our data, Dr. Yang Xia at University of Texas Houston identified a key oxygen transport pathway that is upregulated with acclimatization and can be pharmaceutically stimulated by the drug metformin.

To briefly explain our reasoning, it is important to understand that oxygen uptake and release by red blood cells (erythrocytes) relies on sophisticated regulation of hemoglobin's affinity for oxygen by allosteric modulators. One of the best-known allosteric modulators is 2,3-bisphophosphoglycerate (2,3-BPG). Earlier studies demonstrated that erythrocyte 2,3-BPG levels are elevated at high altitude and facilitate oxygen delivery to tissues.(8, 30) However, factors responsible for 2,3-BPG induction at high altitude are unknown. Based on our study of 21 individuals who rapidly ascended and acclimatized to 17,250 feet, we observed three important facts: 1) Plasma adenosine and erythrocyte 2,3-BPG levels were significantly elevated in humans on the first day at high altitude compared to sea level; 2) Adenosine and 2,3-BPG levels were increased further after 16 days of acclimatization at high altitude; 3) There was a strong correlation between the elevations in adenosine and erythrocyte 2,3-BPG levels across the study. These results imply that adenosine might be responsible for 2,3-BPG induction at high altitude. Next, we performed *in vitro* experiments on cultured human erythrocytes to determine if and how adenosine might trigger the increase in 2,3-BPG. We found evidence that adenosine signaling via A_{2B} adenosine receptors (ADORA2B) induced 2,3-BPG production in a protein kinase A (PKA) and AMP activated protein kinase (AMPK)-dependent manner. These studies lead us to believe that upregulation

of this pathway will increase erythrocyte 2,3-BPG, facilitate oxygen delivery to the tissue, and thereby stimulate acclimatization (Figure 4).

Metformin is an FDA-approved drug used to treat diabetic patients by decreasing hyperglycemia, primarily by suppressing glucose production by the liver. Although the molecular basis of metformin action is not fully understood, it is well known to activate PKA and AMPK.(6, 60) Based on our initial observation that PKA and AMPK are essential regulators of 2,3-BPG induction and promoting oxygen release from erythrocytes, we believe that metformin may stimulate a key process of acclimatization and thereby confer protection from high-altitude illnesses and improve SOF performance.

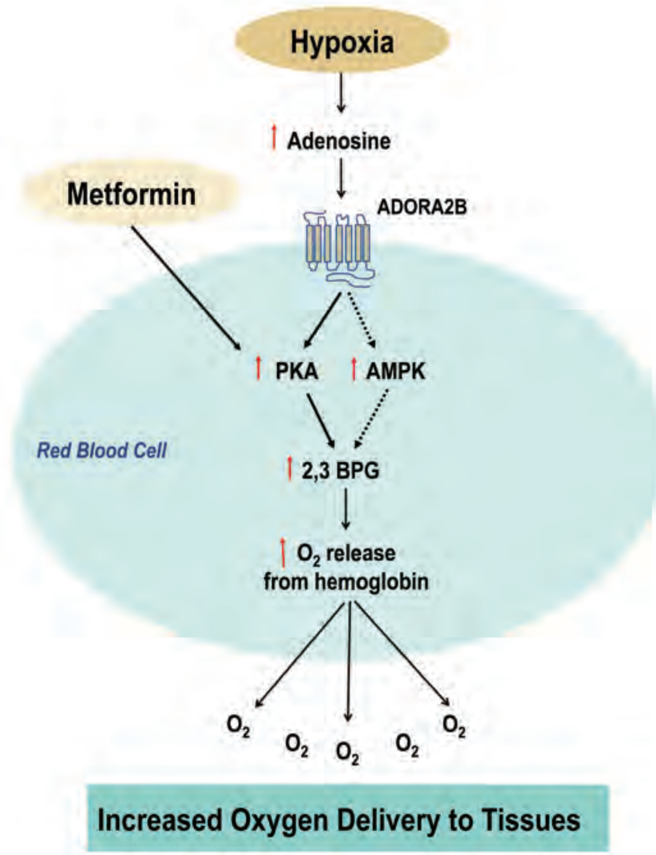


Figure 4. *Mechanism by which metformin may stimulate increased oxygen delivery to promote acclimatization.*

The effect of metformin on exercise performance in healthy, non-diabetic individuals has received little attention.(7, 34) Peak workload achieved during exhaustive cycling tests was not different from placebo, but in one study oxygen consumption was slightly less on metformin.(7) These results suggest that metformin improves mechanical efficiency (more power output for a given amount of oxygen consumption) during high-intensity exercise. We find this effect intriguing because similar improvements in mechanical and mitochondrial efficiency have been reported to explain increases in exercise performance with acclimatization.(25, 35, 50) Additionally, metformin therapy has recently been shown to increase cognitive function in diabetic patients.(19, 37) These results support the neuro-protective effects of metformin reported in animals and cell cultures.(44, 58) **Based on similarities between metformin's mechanism of action and the molecular processes of acclimatization, we believe metformin will be an effective aid to reduce high-altitude illness and improve performance at high altitude.**

Again, we understand that USSOCOM is interested in solving problems today so that SOF can benefit immediately. We believe these three compounds are the most promising agents for preventing high-altitude illness and improving physical and cognitive performance based on our extensive experience in the field. We are ready to begin testing these compounds immediately using a proven and efficient field study design described below.

Why choose us?

Our team of investigators has extensive experience in all aspects of conducting military-relevant field experiments with human volunteers. Our experience includes identifying key research questions, obtaining local and DoD regulatory approval in a timely fashion, and rapidly collecting data and disseminat-

ing our findings. In a recent DoD Telemedicine and Advanced Technology Research Center (TATRC) funded study, we took 21 volunteers from Eugene, Oregon to Mt. Chacaltaya, Bolivia (17,250 feet) for a four-month-long investigation of the molecular, cellular, and physiological processes of high-altitude acclimatization. In another DoD-TATRC funded study, we studied 164 volunteers who were recruited from Dallas, Texas and flown to Breckenridge, Colorado over a weekend to identify genetic signatures capable of predicting individual susceptibility to AMS. And, in a current Defense Advanced Research Projects Agency (DARPA)-funded study, we are studying the effects of high altitude on subtle changes in cognitive function. We have a long and productive track record in high-altitude research, with over 200 publications in the field among the principal scientists. Our work is supported by strong connections to the SOF community through ongoing collaborations with the US Army 10th Special Forces Group (Airborne) Tactical Human Optimization Rapid Rehabilitation and Reconditioning (THOR3) at Fort Carson, Colorado, CMSGT Rod Alne, USAF (retired), and The Peak training group in Butte, Montana (see letters of support). Taken together, our capability to address the problems raised in this USSOCOM proposal are second-to-none.

2. Hypotheses

To identify new and effective pharmacological strategies to prevent illness and improve physical and cognitive performance of SOF during high-altitude deployment, we will test three hypotheses.

Hypothesis 1: Quercetin, nifedipine+methazolamide, and/or metformin will reduce the incidence and severity of AMS over three days at altitudes between 10,000 and 13,000 feet.

Hypothesis 2: Quercetin, nifedipine+methazolamide, and/or metformin will improve physical performance over three days at altitudes between 10,000 and 13,000 feet.

Hypothesis 3: Quercetin, nifedipine+methazolamide, and/or metformin will improve cognitive performance over three days at altitudes between 10,000 and 13,000 feet.

3. Technical Objectives

This proposal has three main technical objectives to test the effectiveness of three novel compounds to improve SOF performance at high altitude in a rigorous scientific manner.

Technical Objective 1: To test quercetin in 15 young, healthy men for reduction in the incidence of high-altitude illnesses, enhanced physical performance, and better cognitive function at high altitude.

Technical Objective 2: To test nifedipine+methazolamide in 15 young, healthy men for reduction in the incidence of high-altitude illnesses, enhanced physical performance, and better cognitive function at high altitude.

Technical Objective 3: To test metformin in 15 young, healthy men for reduction in the incidence of high-altitude illnesses, enhanced physical performance, and better cognitive function at high altitude.

Details of the methods we will employ to achieve these technical objectives are given below.

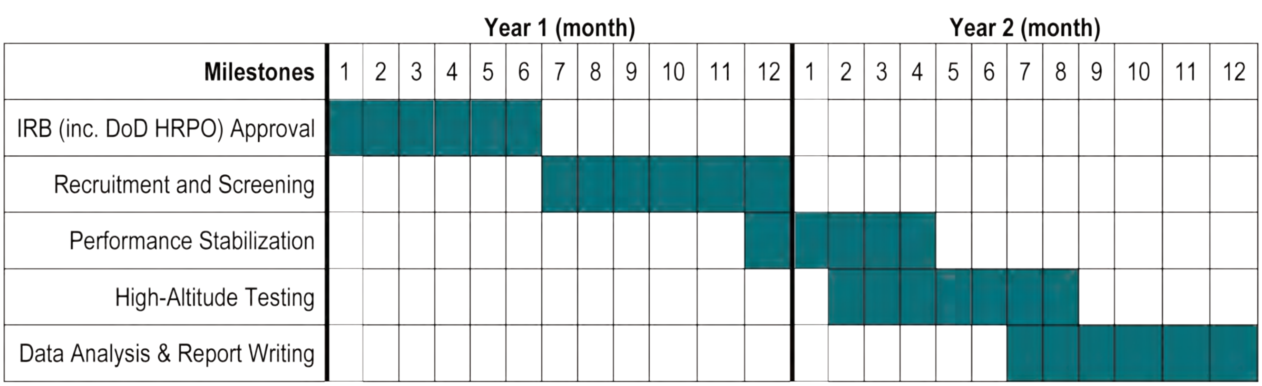
4. Project Milestones

This ambitious plan is fully realizable because of our recent experience conducting DoD-TATRC-funded field studies. In those studies, we tested whether measurements of gene expression at sea level could predict who would later develop AMS. In the first of two field trials for that study, we could predict which young, healthy civilians would either get sick with AMS or stay healthy with 90% accuracy. The validation of that study was just completed and analyses are underway. If successful, a test will be developed for broad military and civilian use to predict at sea level susceptibility of AMS. The third study was a massive effort to study 21 subjects at sea level and after several weeks at high altitude at 17,250 feet in Bolivia. The results form that study are the first description of the molecular mechanisms responsible for the human body's response to prolonged hypoxia and have led us to propose metformin as a novel candidate drug for improving human performance at high altitude. We conducted both of these studies at the same time, obtained Institutional Review Board (IRB) and Human Research Protection Office (HRPO) clearance for both in a timely manner, and have met all milestones for the proposed studies. This success gives us great confidence that we can complete the proposed studies as outlined here.

The total timeline for this project is 24 months (see below). Six months for project startup and IRB + HRPO approvals, 12 months for field data collection, 6 months for data analysis and report writing. This schedule allows time for schedule slippage if DoD HRPO is delayed in processing IRB approval and allows ample time for our experienced team to recruit subjects, conduct field studies, and analyze data.

We will deliver quarterly interim progress reports. At the completion of 18 months, we will deliver preliminary results from the field trials. At 24 months we will present a final report including analysis of all results from all trials, a presentation suitable for wide distribution of the final results, and executive recommendations for implementations of the findings for immediate USSOCOM application.

Complete project timeline



5. Military Significance

US fighting strategies often require the rapid deployment of personnel into extreme environmental conditions including high-mountainous regions, such as in Afghanistan, Pakistan and Iran. For example, while supporting Operation Enduring Freedom in Afghanistan, troops frequently performed combat operations at moderate (>6,000 feet) to high altitudes (>12,000 feet). Afghanistan has 49% of its landmass above 6,500 feet and the major strategic passes range from 8,800 to over 16,000 feet. The necessity for accelerated deployment prohibits time for gradual altitude acclimatization required for optimal high-altitude military performance. Up to 80% of troops rapidly exposed to altitudes of higher than 16,000 feet will develop AMS.(53) The high incidence of and debilitation caused by AMS poses significant risks

to individuals, units and overall military performance and increases the likelihood of emergency evacuation or in-field AMS treatment costs. The potentially punishing impact of high altitude on troops has been well described. In one study from Operation Enduring Freedom, an estimated 12% of medevacs and hospital admissions were due to severe AMS.(43)

Regardless of the length and size of military deployments in Afghanistan, other high altitude areas (including South America, Iran, and Pakistan) pose possible US security concerns that could put SOF at risk for high altitude-induced illness in the future. **Our project will 1) simulate a military-specific scenario (rapid ascent, high physical activity) at operationally relevant altitudes in Colorado (10,000 to 13,000 feet), and 2) determine the efficacy of three compounds to prevent AMS and improve physical and cognitive function at high altitude.** If any of these drugs show a demonstrable advantage, this will be the first major advance in this field for more than 25 years. **Because we are ready to test these compounds today, SOF may benefit from this study tomorrow.**

6. Public Purpose

Acute mountain sickness (AMS) affects > 25% of unacclimatized visitors to altitudes between 7,000 and 9,000 feet.(22) The risk increases substantially at higher altitudes, reaching 42% at 10,000.(11) Given the millions of visitors to high altitudes in the United States and abroad each year, AMS is considered a public health problem.(20) No new drugs to help prevent or treat AMS have been developed for the past 25 years. Those that are currently available only work moderately well or are associated with many undesirable side effects and risks. We propose to test three novel pharmacological approaches to preventing AMS. Quercetin, an over-the-counter nutraceutical, and nifedipine+methazlamide, two drugs already approved for use in humans, are effective for preventing HAPE/HACE and may improve physical and cognitive performance at high altitude. Metformin, a drug commonly used to treat diabetes, induces biochemical changes recently linked to successful acclimatization and protection from high-altitude illnesses. In addition, any of these drugs that are proven effective at high altitude may then be tested for application ot diseases at low altitude that impact lung oxygen levels, including some heart and lung diseases. We propose one or more of these approaches will substantially improve SOF health and performance at high altitude.

7. Methods

We will follow a standard experimental model (double-blind, placebo controlled, matched cohort design) to test the hypotheses that quercetin, nifedipine + methazolamide, and/or metformin will prevent AMS and improve physical and cognitive performance during a simulated three-day deployment to high altitude (10,000 to 13,000 feet; Figure 5).

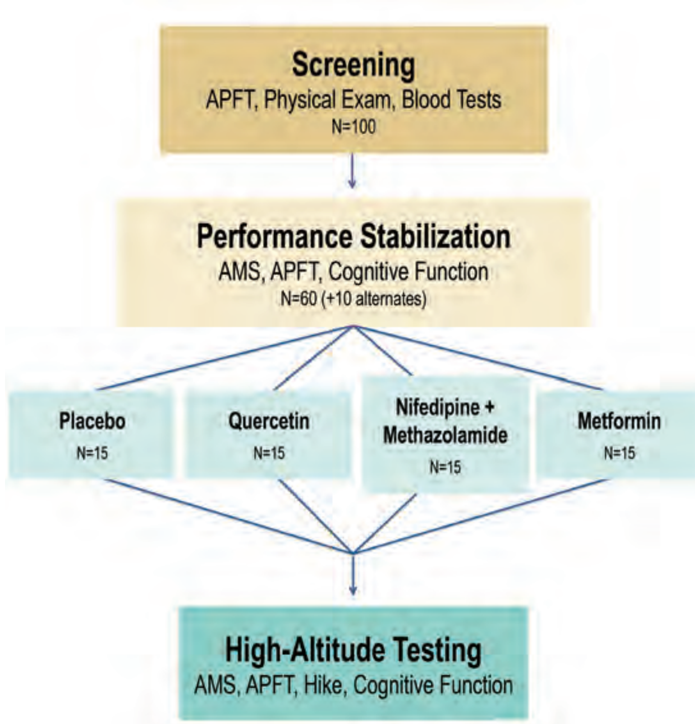


Figure 5. *Study outline using a double blind, placebo controlled, matched cohort design.*

Who will be studied?

We propose to study civilians who meet the basic health and physical fitness eligibility standards for SOF. Subjects will be matched and assigned to the four treatment groups to achieve the necessary statistical power to detect meaningful changes in health and performance at altitude. Our team has extensive experience in recruiting and screening subjects on college campuses to achieve this goal. Our recruitment and group assignment plan is described below.

Subject Recruitment

Following approval from local IRBs and DoD HRPO, 100 men will be recruited from student populations near sea level in central Michigan (Alma College, Central Michigan University, Michigan State University). The major inclusion criteria will be: healthy men 18-30 years old who can meet physical fitness requirements for SOF training outlined by the US Army (see below). The major exclusion criteria will be: women; those with anemia; those with known disease; those with a history of significant head injuries or migraines; those who are unable to achieve the minimum physical fitness standards; those taking any medications that interfere with oxygen delivery and transport (including sedatives, sleeping aids, tranquilizers, diuretics, alpha and beta blockers, and any medication that depresses ventilation), and those with known allergies to sulfonamide-based drugs. All potential subjects will give written informed consent prior to the competitive selection procedures listed below.

Physical Screening

Eligible volunteers consenting to the protocol will undergo physical examinations, including blood draws for standard blood chemistry, and perform the Army Physical Fitness Test (APFT) to verify health and fitness standards required of SOF. We have recently used these tests to successfully and efficiently screen hundreds of volunteers for two DoD funded studies. To meet the higher standards required of SOF, more stringent selection criteria will be applied. Specifically, all participants must be able to score a minimum of 60 points based on each of the three APFT criteria for 17 to 21 year olds (42 push ups, 53 sit-ups, 2-mile run in <15:54). Usually the APFT score is age-adjusted, getting ‘easier’ with age, but by eliminating the age-adjustment we will ensure that all subjects meet the highest minimum standards. Additionally, we will give preference to those who score highest on the APFT. Those achieving total scores >240 points will be given first preference for enrollment. Those scoring between 229-240 points will be given second preference. Those scoring between 180 and 228 points will be given third preference.

Performance Stabilization

To ensure accurate baseline measurements of physical and cognitive performance, the top 60 men (and 10 alternates) with the most similar physical characteristics will repeat the APFT, a computerized battery of cognitive function tests, and AMS symptom questionnaires at least twice at sea level. This strategy will minimize the influence of learning effects associated with the tests and facilitate matching between groups.

Group Assignments

The top 60 men will be matched according to height, weight and best APFT scores to form 15 sets of four subjects who have similar baseline characteristics. From these sets we will randomly assign subjects to the four treatment groups: placebo, quercetin, nifedipine+methazolamide, and metformin (15 in each group). This matching process will ensure that the baseline performance of those in the placebo group is similar to those in the experimental groups and increase our ability to detect meaningful effects.

How will the effectiveness of the treatments be determined?

The experimental procedures have been designed to resemble the demands that SOF experience during rapid deployment to rugged mountainous environments. We have successfully conducted several field studies using a similar design and favor this practical field approach to less relevant laboratory studies.

Drug Administration

Subjects will begin taking oral medications 48 hours prior to their scheduled departure from sea level and continue treatment through their stay at high altitude. All compounds will be prepared and coded by a clinically licensed pharmacy. Investigators and subjects will be blinded to the identity of the compounds until after the study is completed. Those in the quercetin group will take 500 mg twice daily. This matches the therapeutic dosage used in studies showing positive effects on physical performance(10, 33, 38) and is not associated with any known side effects.(18, 52) Those in the nifedipine+methazolamide group will take 30 mg of sustained release nifedipine and 125 mg of methazolamide twice daily to achieve therapeutic doses reported in the literature.(5, 59) Those in the metformin group will take 500 mg once a day for 48 hours, then 500 mg twice daily while at altitude. This uptitration schedule is commonly used to minimize potential gastrointestinal discomfort.(7, 45) Those in the placebo group will take a non-physiologically active substance (cellulose) packaged in identical capsules.

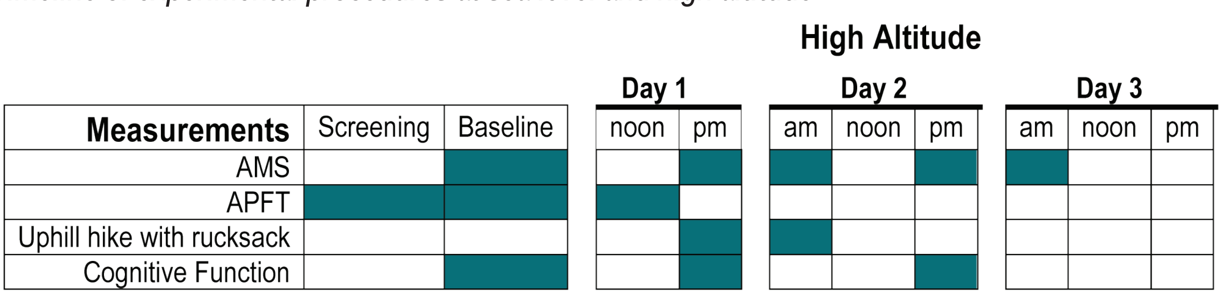
High-Altitude Testing

Subjects will be transported to Denver, Colorado in groups of 10-20 by commercial airlines and then immediately driven to Breckenridge, Colorado by charter buses. The total travel time from sea level to high altitude will be ~6 hours. Subjects will follow a strict regimen of tasks designed to simulate SOF-relevant physical activity at high altitude (10,000 to 13,000 feet) over two days and two nights before returning to Michigan on the 3rd day. Based on our previous study of 164 subjects, this ascent profile reduced APFT 2-mile run performance by ~2 min (13%) and induced a peak incidence of AMS in ~50% of the subjects on the first evening at high altitude. The large decrements in performance and high incidence of AMS associated with this protocol increase the likelihood of detecting significant and meaningful positive effects from the experimental compounds.

What will be measured and when?

Repeated trials of all study measurements at sea level will ensure stability of performance and equivalence between groups prior to travel. At high altitude, the effectiveness of the treatments on four key outcomes will be assessed: 1) APFT scores on arrival, 2) AMS, and 3) cognitive function scores on the first evening and 4) uphill hike with rucksack time on the 2nd morning at high altitude. Additionally, we will assess the time course of acute changes in AMS and cognitive function at high altitude. Timing of all respective tests is shown in the following timeline. Details of each test are described below.

Timeline of experimental procedures at sea level and high altitude



AMS Scoring

The incidence and severity of AMS will be determined using a subset of the Environmental Symptoms Questionnaire (ESQ) and the Lake Louise AMS Scoring System (LLSS), the two most common and accepted measures of AMS. The ESQ is a self-reported, 68-question inventory used to document symptoms induced by altitude and other stressful environments.(49) A weighted average of scores from 9 symptoms (headache, lightheaded, dizzy, etc.) designated AMS-C will be calculated. AMS-C scores greater than 0.7 are considered positive for AMS. The LLSS consists of a six question self-reported assessment of AMS symptoms, with a score of $\geq$3, including headache, defined as AMS.(48) The altitude-illness assessment questionnaires will be administered on paper forms and tabulated in digital format at the end of each day. After completing the AMS assessment forms, arterial saturation will be monitored by pulse oximetry to monitor overall adjustment level to high altitude and screen for development of severe altitude illness. Each AMS assessment and noninvasive pulse oximetry measurement will take ~ 10 min to complete. These measurements will be made twice at sea level, immediately upon arrival at high altitude, and every night and morning during the stay in Colorado.

APFT

The APFT will be used to assess overall aerobic fitness due to its military relevance.(27) All testing will be performed on a standard 400-meter track in Alma, Michigan and in Breckenridge, Colorado. Briefly, subjects will be led through a 15 minute warm up by trained staff and scored based on the maximum number of pushups performed in 2 minutes, the maximum number of sit ups performed in 2 minutes, and the time for a competitive 2-mile run. The purpose of this test battery is two-fold. First, it allows us to select subjects based upon SOF-relevant standards. Second, it provides a standardized assessment of physical performance at high altitude. The APFT will be performed twice at sea level and once immediately upon arrival at high altitude.

Uphill Hike with Rucksack

We previously developed this test to simulate military style field operations for another DoD funded study. Subjects will be asked to complete a 3.1-mile uphill hike with a 35-pound rucksack. The course follows a rugged hiking/jeep trail that begins in a wooded area at 10,627 feet and ends on a ridge above tree line at 12,595 feet. Subjects will hike the course as a group after the APFT on the first day at high altitude to familiarize themselves with the terrain. The following morning (2nd day) they will be asked to complete the course as fast as possible while being timed. Fit subjects, free of AMS, can finish the course in ~60 minutes.

Cognitive Function

We will use the Defense Automated Neurobehavioral Assessment (DANA) to assess neurocognitive function across the study. We have recently documented decrements in several components of the DANA during a simulated rapid deployment to high altitude that improved with acclimatization(53) and thus believe the tests are sensitive and specific to the cognitive challenges SOF face in this environmental extreme. Using a handheld computer, the following nine cognitive function tests will be administered:

1) Simple Reaction Time-1 (measured at the beginning of neurocognitive testing to gain an understanding of pure visual-motor response);
2) Simple Reaction Time-2, repeated at the end of neurocognitive testing to assess diminished reserve of cognitive effort on reaction time;
3) Procedural Reaction Time, a measure of choice reaction time and accuracy;
4) Go-No-Go, a measure of speed, accuracy and impulsivity;
5) Code Substitution—Simultaneous, a measure of visual scanning and attention, learning, and

immediate recall of digit-symbol pairings;

6) Code Substitution—Delayed Recall, a measure of short-term memory for digit-symbol pairings;

7) Spatial Discrimination, a measure of visuospatial analytic ability;

8) Matching to Sample, an assessment of attention and memory for visuospatial discrimination;

9) Sternberg's Memory Search, a measure of working memory for letters.

The total time required to complete the battery of tests is ~20 minutes. DANA will be administered twice at sea level and in the evening of the 1st and 2nd days at high altitude.

How will the data be analyzed?

The three primary outcomes of AMS, cognitive function, and physical performance obtained at high altitude will be analyzed using one-way ANOVA with planned comparisons to determine if those in each of the experimental treatment groups performed better than those in the placebo group (see Figure 6). This method of analysis considers variance across all treatments, but controls for type I error ($\alpha = 0.05$). Chi square analysis will be used to evaluate the incidence of AMS. An *a priori* power analysis based on the expected incidence of AMS in the placebo (50%) and experimental (0%) treatments, revealed that 14 subjects per group would be necessary to detect a meaningful positive outcome. Similarly, using the average APFT 2-mile run time at high altitude from our previous study (16:40 ± 2:10 minutes:seconds), 10 subjects per group would be necessary to detect a 4% improvement in performance (~40 seconds) if the drugs have a moderate to large effect (Cohen's d = 0.70, $\alpha = 0.05$). Assigning 15 subjects to each group (N=60) is thus expected to maintain statistical power at 80%.

Summary

There have been no significant advancements in improving human health and performance at high altitude in the last 25 years. This double-blind, placebo controlled, matched cohort study is well designed to test the efficacy of three new pharmacological strategies to improve SOF health and performance at high altitude. As summarized in Figure 6, the three approaches are independent, but each has significant potential to transform SOF performance at high altitude. Our team is uniquely qualified and prepared to test these strategies today, so that SOF may reap the benefits tomorrow.

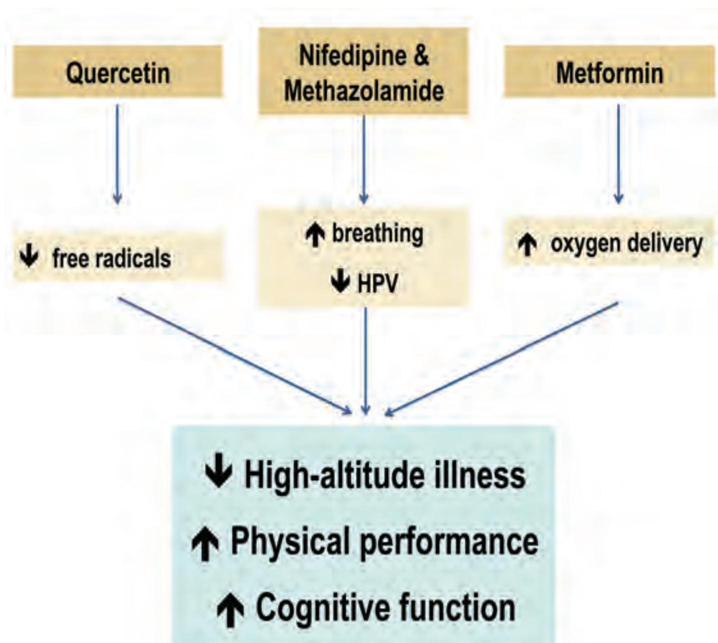


Figure 6. *Summary graphic of overall study design testing three novel compounds for improving SOF performance at high altitude via three completely unique and independent mechanisms. (HPV= hypoxic pulmonary vasoconstriction).*

Glossary of Abbreviations

2,3-BPG- 2,3-Bisphosphoglycerate
ADORA2B- A_{2B} adenosine receptors
AMPK- Adenosine monophosphate kinase
AMS- acute mountain sickness
ANOVA- analysis of variance
APFT- Army Physical Fitness Test
DANA- Defense Automated Neurobehavioral Assessment
DARPA- Defense Advanced Research Projects Agency
DoD- Department of Defense
ESQ- Environmental Symptoms Questionnaire
FDA- Food and Drug Administration
HACE- high-altitude cerebral edema
HAPE- high-altitude pulmonary edema
HRPO- Human Research Protection Office
IRB- Institutional Review Board
LLSS- Lake Louise Scoring System
O_2- oxygen gas
pH- negative logarithm of concentration of hydrogen ions
PKA- protein kinase A
SOF- Special Operations Forces
TATRC- Telemedicine and Advanced Technology Research Center
US- United States
USSOCOM United States Special Operations Command

ALMA COLLEGE

July 28, 2014

To: Dr. Robert Roach

From: Dr. John Davis

Re: Letter of Support fo*r "Three New Ideas to Protect Special Forces from the Stress of High Altitude " by Dr. Robert Roach, PI.*

I am pleased to support the proposal to investigate the testing of three novel compounds to enhance human performance at high altitudes. I can attest to the importance of this project to the general field of high altitude medicine and physiology and more importantly to it's potential to provide important new insights into this area. Clearly the approaches proposed in this study would improve both physical and cognitive performance while protecting the warfighter from high-altitude illnesses. That would present a major breakthrough!

Working with you and your team last year in the DoD-funded study for AMS prediction really convinced me that collaborating together on this project would produce an even better study using our sea level laboratory combined with your field operation in Colorado. All of my team here in Michigan is excited for the opportunity to work together on this project. As outlined in the formal subcontract documents, we will recruit the subjects and work with you to meet all regulatory approval requirements.

I look forward to working with you and your team if this project gets funded. In particular, I look forward to contributing my expertise to this exciting project!

Sincerely,

John E Davis

Dr. John E. Davis
Charles A. Dana Professor
NSF-PRISM Director
Department of Integrative Physiology and Health Science
Alma College
Alma, MI 48801
989-463-7158 davisj@alma.edu

July 28, 2014

To: USSOCOM, U.S Department of Defense

From: Paul Goldberg MS, RD, CSCS, CSSD – Human Performance Program Coordinator – 10th Special Forces Group Tactical Human Optimization Rapid Rehabilitation and Reconditioning (THOR3)

Re: Letter of Support for *"Three New Ideas to Protect Special Forces from the Stress of High Altitude"*

I am pleased to support the proposal to investigate the use of quercetin, nifedipine+methazolamide, and metformin to enhance human performance at high altitudes. I have collaborated with Dr. Andrew Subudhi from the University of Colorado on a number of educational projects related to soldier performance over the past four years. In this capacity, I have learned of the Altitude Research Center's recent advancements in the field and believe they have identified three promising nutraceuticals/pharmaceuticals that may improve physical performance upon rapid deployment to high altitude. If successful, these compounds would mark the most significant advancement in improving physical performance at high altitude in the past several decades.

As a consultant on the current project my role will be to provide advice and assistance in making the research as field-applicable as possible. With over twenty years of experience as a strength coach and Registered Dietitian for the US Army Special Forces, Navy Special Warfare Development Group, Colorado Avalanche National Hockey League Club, and NCAA Division I athletic programs, I bring a wealth of expertise to this problem, particularly related to field-testing at high altitudes. If this project is funded, I will consult on conducting the extensive field-testing proposed in this project to assure subjects recruited meet the physical profile expected of special operation forces.

I look forward to contributing to this exciting project, and for another opportunity to work with the talented scientists at the University of Colorado.

Sincerely,

Paul Goldberg MS, RD, CSCS, CSSD

111 Airport Road, Butte, MT. 59701
406-494-7999

July 28, 2014

To: Dr. Robert Roach

From: Mr. Rod Alne

Re: Letter of Support for *"Three New Ideas to Protect Special Forces from the Stress of High Altitude " by Dr. Robert Roach, PI.*

I am pleased to support the proposal to investigate the testing of three novel compounds to enhance human performance at high altitudes. I can attest to the importance for SOF to have some new ideas in this area, especially an approach that would improve both physical and cognitive performance while protecting from high-altitude illnesses. That would present a major breakthrough!

I can personally attest to the issues of altitude on our SOF personnel. I was a SOF member for 27 years and deployed in Afghanistan twice, since retiring in 2005. Due the issues, I saw and the lack of knowledge dealing with altitude I stated The Peak Inc. in 2005 located in SW Montana. This mission of The Peak Inc is to educate and apply a real world experience to our SOF members dealing with altitude and it's effects prior to deployment.

I look forward in working with you and your team if this project gets funded. In particular, I would bring my real world SOF experience to the consulting with you on the design of the field experiments. Though there are real world limits as to what can be simulated in civilians in the field, you and your team have demonstrated a strong ability to conduct state of the art and militarily-relevant human research. You are the best at what you do. Working together again would be a pleasure.

I look forward to contributing to this exciting project!

Sincerely,

Rod Alne, President
The Peak Inc.

University of Colorado Denver

David Irwin, PhD
Cardiovascular Pulmonary Research Laboratory
Anschutz Medical Campus, RC-II, Box B 133
Aurora, CO 80045
(303) 724-3684
David.Irwin@UCDenver.edu

July 28, 2014

To: Dr. Robert Roach

From: Dr. David Irwin

Re: Letter of Support for *"Three New Ideas to Protect Special Forces from the Stress of High Altitude " by Dr. Robert Roach, PI.*

I am pleased to support the proposal to investigate the testing of three novel compounds to enhance human performance at high altitudes. I can attest to the importance in the general field of high altitude medicine and physiology to have some new ideas in this area, especially an approach that would improve both physical and cognitive performance while protecting from high-altitude illnesses. That would present a major breakthrough!

I am please to see our recent work from our DARPA-funded effort to identify in animal models novel combinations of drugs that would prevent fluid leak in brain and lung tissue being used in this proposal. One of the most promising drug combinations was nifedipine + methazolamide. I am pleased to see the rapid turn around of our preclinical tests translate to a human evaluation of the actions of this drug combination to improve SOF performance at high altitude.

I look forward in working with you and your team if this project gets funded. In particular, I will bring my experience with preclinical animal models, pharmacokinetics experimental design, data from all our previous proof of principle pharmacological experiments in animal models of high altitude illnesses to bear on refining the approach proposed in your experiments; and to continue to iterate between pre-clinical and clinical testing.

I look forward to contributing to this exciting project!

Sincerely,

David Irwin, PhD
Assistant Professor Division of Cardiology
University of Colorado Denver

12700 E. 19th Avenue, Mail Stop B133, Research 2, Aurora, Colorado 80045
Phone: 303-724-3684, David.Irwin@UCDenver.edu

THE UNIVERSITY *of* TEXAS
HEALTH SCIENCE CENTER AT HOUSTON
MEDICAL SCHOOL

Medical School
Department of Biochemistry
and Molecular Biology

July 28, 2014

To: Dr. Robert Roach

From: Dr. Yang Xia, M.D., PhD.

Re: Letter of Support for *"Three New Ideas to Protect Special Forces from the Stress of High Altitude " by Dr. Robert Roach, PI.*

I am pleased to support the proposal to investigate the testing of three novel compounds to enhance human performance at high altitudes. I can attest to the importance in the general field of high altitude medicine and physiology to have some new ideas in this area, especially an approach that would improve both physical and cognitive performance while protecting from high-altitude illnesses. That would present a major breakthrough!

I am pleased to see our recent work together from your DoD-funded study on human acclimatization to high altitude being brought to bear already on a trial of the effectiveness of metformin. All evidence we have suggests that this will be a productive approach by mimicking the effects of acclimatization.

I look forward in working with you and your team if this project gets funded. In particular, I would bring my experience with the basic mechanisms of how metformin would work on the adenosine and 23BPG pathway to improve performance in hypoxia. In addition, our pending proposals for extensive animal testing of these and other ideas to manipulate oxygen delivery to the tissue can continue to feed into clinical evaluations at relevant high altitudes.

I look forward to contributing to this exciting project!

Sincerely,

Yang Xia

Yang Xia, M.D., Ph.D.
Professor
Biochemistry and Molecular Biology Department
UT-Houston Medical School

Environmental Compliance Assurance

The offeror currently X IS __ IS NOT (*check appropriate category*) in compliance with applicable national, state, and local environmental laws and regulations. (If not in compliance, attach details and evidence of approved mitigation measures.) The offeror has examined the activities encompassed within the proposed action for compliance with environmental laws and regulations. (*Enter proposal title*)

"THREE NEW IDEAS TO PROTECT SPECIAL FORCES FROM THE STRESS OF HIGH ALTITUDE"

The offeror states that the conduct of the proposed action:

1. WILL NOT violate any applicable national, state, or local environmental law or regulation, and
2. WILL NOT have a significant impact on the environment.

The offeror agrees that if the work required under the proposed action at any time results in a significant impact on the environment or a violation of any applicable environmental law or regulation, the offeror will immediately take appropriate action, to include notifying and/or coordinating with the appropriate regulatory agencies as required by law and notifying the Grants Officer.

ETHAN CARTER
Name of Official Responsible for Environmental Compliance (Printed)

DIRECTOR ENVIRONMENTAL HEALTH & SAFETY
Title of Official Responsible for Environmental Compliance (Printed)

[signature] 7 JULY 2014
Signature Date

UNIVERSITY OF COLORADO DENVER
Name of Organization (Printed)

ORGANIZATIONAL DATA

Organization: The University of Colorado Denver

Federal Identifier/Log No (if available):

Project Title: Three new ideas to protect special forces from the stress of high altitude

Principal Investigator: Roach Roach, Ph.D.

Primary Place of Performance: Enter city, state, zip code, or enter country if outside of the U.S.
Aurora, CO 80045

1. Complete the following codes, as applicable:

    DUNS 0410963140000

    CAGE 0P6C1

    TIN 84-6000555

    FICE 004508

2. The organization, by checking all applicable boxes, represents that it operates as:

State Government	Indian Tribe
County Government	Private Higher Educational Institution
Municipal or Township	Individual
Special District Government	Profit Organization (Not a small business)
Independent School District	Small Business
Nonprofit Agency (Other than Educational)	All Other
✓ State Controlled Institute of Higher Education	

3. In addition, indicate if any of the following apply:

    Historically Black College and University

    Minority Institution

    Foreign University

    Foreign Nonprofit Organization

    Federally Funded R&D Center (Academic)

    Federally Funded R&D Center (Nonprofit)

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: University of Colorado Denver

* Street1: 12469 East 17th Place, Building 400 Street2: Mail Stop F524
* City: Aurora County: Adams * State: CO: Colorado
Province: * Country: USA: UNITED STATES * Zip / Postal Code: 80045-2571

Project/Performance Site Location 1

Organization Name: Alma College

* Street1: 614 West Superior Street Street2:
* City: Alma County: * State: MI: Michigan
Province: * Country: USA: UNITED STATES * Zip / Postal Code: 48801-1599

	File Name	Mime Type
Additional Location(s)		

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0410963140000
*** Budget Type:** ● Project ❍ Subaward/Consortium
Enter name of Organization: University of Colorado Denver

*** Start Date:** 12-01-2014 *** End Date:** 11-30-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Robert	Corwine	Roach Jr.		PD/PI	182,703.00	3			45,676.00	12,789.00	58,465.00
2.		Andrew	W.	Subudhi	Ph.D.	Co-PD/PI	99,153.00			3	24,788.00	6,941.00	31,729.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **90,194.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates	1.77			6,257.00	1,189.00	7,446.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12			43,538.00	12,191.00	55,729.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**63,175.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**153,369.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0410963140000
*** Budget Type:** ● Project ❍ Subaward/Consortium
Enter name of Organization: University of Colorado Denver

*** Start Date:** 12-01-2014 *** End Date:** 11-30-2015 **Budget Period: 1**

C. Equipment Description
List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,500.00
2. Foreign Travel Costs	
Total Travel Cost	**3,500.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0410963140000
*** Budget Type:** ● Project ❍ Subaward/Consortium
Enter name of Organization: University of Colorado Denver

*** Start Date:** 12-01-2014 *** End Date:** 11-30-2015 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	5,875.00
2. Publication Costs	0.00
3. Consultant Services	7,500.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	64,533.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	40,762.00
Total Other Direct Costs	**118,670.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**275,539.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. MTDC	55	148,087.00	81,448.00
2. MTDC	55.5	87,919.00	48,795.00
		Total Indirect Costs	**130,243.00**

Cognizant Federal Agency DHHS, Wally Chan, 415-437-7829
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**405,782.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: budget_justification_per1.pdf Mime Type: MIMETYPE
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0410963140000
*** Budget Type:** ● Project ❍ Subaward/Consortium
Enter name of Organization: University of Colorado Denver

*** Start Date:** 12-01-2015 *** End Date:** 11-30-2016 **Budget Period: 2**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Robert	Corwine	Roach Jr.		PD/PI	188,184.00	2.4			37,637.00	10,538.00	48,175.00
2.		Andrew	W.	Subudhi	Ph.D.	Co-PD/PI	102,128.00			2.4	20,426.00	5,719.00	26,145.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **74,320.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates	2.35			8,593.00	1,633.00	10,226.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	7.8			29,149.00	8,162.00	37,311.00
1	Physician	0.54			9,386.00	2,628.00	12,014.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**59,551.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**133,871.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0410963140000
*** Budget Type:** ● Project ❍ Subaward/Consortium
Enter name of Organization: University of Colorado Denver

*** Start Date:** 12-01-2015 *** End Date:** 11-30-2016 **Budget Period: 2**

C. Equipment Description
List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,531.00
2. Foreign Travel Costs	
Total Travel Cost	**5,531.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0410963140000
*** Budget Type:** ● Project ❍ Subaward/Consortium
Enter name of Organization: University of Colorado Denver

*** Start Date:** 12-01-2015 *** End Date:** 11-30-2016 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	1,225.00
2. Publication Costs	3,000.00
3. Consultant Services	7,500.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	37,759.00
Total Other Direct Costs	**49,484.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**188,886.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. MTDC	55.5	188,885.00	104,831.00
		Total Indirect Costs	**104,831.00**

Cognizant Federal Agency DHHS, Wally Chan, 415-437-7829
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**293,717.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: budget_justification_per1.pdf Mime Type: MIMETYPE
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**164,514.00**
Section B, Other Personnel		**122,726.00**
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		**287,240.00**
Section C, Equipment		
Section D, Travel		**9,031.00**
1. Domestic	9,031.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**168,154.00**
1. Materials and Supplies	7,100.00	
2. Publication Costs	3,000.00	
3. Consultant Services	15,000.00	
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	64,533.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	78,521.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**464,425.00**
Section H, Indirect Costs		**235,074.00**
Section I, Total Direct and Indirect Costs (G + H)		**699,499.00**
Section J, Fee		

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0725825050000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Alma College

*** Start Date:** 12-01-2014 *** End Date:** 11-30-2015 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		John		Davis		Subcontract PI	82,890.00	1.3			9,210.00	705.00	9,915.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **9,915.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	Technician	3			6,000.00	460.00	6,460.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**6,460.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**16,375.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0725825050000
*** Budget Type:** ❍ Project ● Subaward/Consortium
Enter name of Organization: Alma College

*** Start Date:** 12-01-2014 *** End Date:** 11-30-2015 **Budget Period: 1**

C. Equipment Description
List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
	Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,500.00
2. Foreign Travel Costs	
Total Travel Cost	**2,500.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0725825050000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: Alma College

*** Start Date:** 12-01-2014 *** End Date:** 11-30-2015 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	3,607.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other Costs	34,750.00
Total Other Direct Costs	**38,357.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**57,232.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Modified Total Direct Costs	48	15,210.00	7,301.00
		Total Indirect Costs	**7,301.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**64,533.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: subaward_justification_per1.pdf (Only attach one file.)	Mime Type: MIMETYPE

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		**9,915.00**
Section B, Other Personnel		**6,460.00**
Total Number Other Personnel	4	
Total Salary, Wages and Fringe Benefits (A+B)		**16,375.00**
Section C, Equipment		
Section D, Travel		**2,500.00**
1. Domestic	2,500.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**38,357.00**
1. Materials and Supplies	3,607.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	34,750.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**57,232.00**
Section H, Indirect Costs		**7,301.00**
Section I, Total Direct and Indirect Costs (G + H)		**64,533.00**
Section J, Fee		

Budget Justification Sub-award Alma

Key Personnel

John Davis, PhD Charles A. Dana Professor of Integrative Physiology at Alma College, Alma, Michigan, Co-Investigator (1.3 calendar months in year 1) will be in charge of screening and recruiting all research subjects. He has done various field projects and his team therefore is well placed to assist in selecting the subjects for this project. His requested salary is 1/9 of salary (based on NSF model)= $9210+ $705 benefits.

Other personnel
A portion of a lab coordinator's time as well as 3 student research assistants will be needed for the project. Total salary $6,000 plus 7.65% benefits = $6,460.

Total Personnel cost = $16,375

Travel: Travel Alma-Denver for John Davis; 5 round trips @$500 per trip = **$2,500**

Other Cost

Subject related:
Recruit and screen 100 subjects with AFT

- Recruiting– newspaper advertising, travel to recruit, etc. = $1,000
- Fitness testing $75 per subject x 100 = $7,500
- Supplies and analysis for screening = $3,607

Subtotal = $12,107

Enroll 70 subjects

- Medical screening including blood draw - $75 per subject x 70 = $5,250
- Subject payments - $300/ subject x 70 = $21,000

Total other expenses = **$38,357**

Budget Justification

KEY PERSONNEL

The research team will be led by **Robert Roach, PhD (3 calendar months in year 1 and 2.4 calendar months in year 2)**, Director, University of Colorado's Altitude Research Center (ARC), a renowned high-altitude physiology research group. The ARC's primary research focus is to examine the effects of acute- and chronic-hypoxia on human health and the pathophysiology of hypoxia-related disease to identify potential prophylactic targets. As the Director at ARC, Dr. Roach supervises an exceptional team of investigators including multiple clinicians, senior researchers, post-doctoral fellows and research associates. Dr. Roach has extensive experience carrying out large-scale physiological research efforts including international collaborations, laboratory, and field studies. Dr. Roach is currently coordinating the final stages of two DOD-funded studies, one on the Omics of acclimatization and the other one on prediction of AMS using a genetic test; he is also conducting the human testing phase of a DARPA study.

Andrew Subudhi, PhD, Co-Principal Investigator (3 summer months in year 1 and 2.4 summer months in year 2) is an Associate Professor of Biology at the University of Colorado at Colorado Springs and holds a joint research appointment within the Department of Emergency Medicine at the University of Colorado Denver. Dr. Subudhi has extensive experience with athletic performance at altitude; he supervised the physiological testing program at the United States Olympic Committee Network Affiliate site in Salt Lake City, Utah, where he was integrally involved with sport science services for US Speed skating and the US Ski and Snowboard Association. He continues to serve as a consultant for these organizations, but currently devotes more time to consulting with USA Cycling in Colorado Springs, CO. As an expert in endurance performance, Dr. Subudhi has received invitational orders to collaborate with the US Army on two large acclimatization studies on Pike's Peak and has been an invited speaker to military sponsored symposia. His expertise is important to this project. He holds a 9-month academic appointment with the University of Colorado at Colorado Springs and collaborates on research projects at the Altitude Research Center for the remainder.

John Davis, PhD, Charles A. Dana Professor of Integrative Physiology at Alma College, Alma, Michigan, Co-Investigator (1.3 months year 1) will be in charge of screening and recruiting all research subjects. Dr. Davis and his team have a great deal of experience with recruiting subjects for field projects. For the past 15 years he has been studying altitude physiology in the mountains of Colorado. These studies involved subject recruitment, screening, and all of the logistics that go along with getting sea level residents from Michigan to high altitude. Most recently, he has led several research projects in the Andes (Ecuador) looking at the physiological adaptations to altitude in high altitude natives. These field studies also involved extensive subject recruitment and screening. As a result of these experiences, Dr. Davis and his team are well placed to assist in selecting and screening the subjects for this project.

His salary is included in the sub award.

Other personnel:
One Postdoctoral Fellow with bio-statistical focus will be working on this project for 1.8 calendar months in year 1 and 2.4 calendar months in year 2. For year 1 a full time Professional Research Assistant (PRA) will be needed; in year 2 their effort will be reduced to 7.8 calendar months. A physician must be present for the field work days. We included the total estimated for physician expenses to amount to 0.6 calendar months, which is included in year 2.
All salaries have been calculated at their levels for the academic year 2014/2015, with a 3% cost of living adjustment per year.
The official fringe benefit schedule has been included below.

<u>TRAVEL</u>

Travel to a National Meeting: Travel to one national meeting (APS – San Diego) for Drs. Roach and Subudhi to present results from this study is requested for both years. Per person costs are: Registration $700, airfare $300 and per diem $200 for 3 days. **Total year 1 and 2 = $3,500**

Travel to USSOCOM coordination meeting in Tampa, FL. for Drs. Roach and Subudhi in year 2. Airfare $543 and per diem $166 for 2.5 days. **Year 2 = $2,331**
Total Travel year 1 = $3,500
Total Travel year 2 = $5,531

OTHER EXPENSES
Note: cost for all field work expenses have been spread equally over both years, since we estimate about half of the subjects will have their altitude visit in year 1 and half in year 2. Costs have also been calculated using a total of 70 subjects, since a margin of 10 subjects must be built in to be guaranteed 60 qualifying participants

1. Materials and supplies
Cost of a blood sampling and analysis in the field is calculated at $35 per subject. **Cost for blood sampling year 1 = (70*$35) * 0.5 = $1,225. For year 2 = $1,225.**
For the fieldwork we will also need to purchase 20 study backpacks to have subjects carry on their weighted run. Cost = $200 (per pack) * 20 = $4,000.
Lastly we will need to purchase a professional quality (SECA or equivalent) scale to precisely measure the packing load for each subject. Estimated cost $650
Total Materials and Supplies cost year 1 = $1,225 + $4,000 + $650 = $5,875
Total Materials and Supplies cost year 2 = $1,225

2. Publication Cost. Average cost for publication preparation and page charges are estimated to be $3,000 per paper. We anticipate publishing our findings in one paper in year 2.

3. Consultant Services
Yang Xia, MD, PhD, Co-Investigator. Responsible for analysis and interpretation of metformin results in comparison with findings in animal and other human studies of adenosine and acclimatization. Her compensation will be $5,000 for the total period or $2,500/year.

David Irwin, PhD, Consultant on translation of DARPA-funded drug discovery for protection from high altitude illness from rodents to humans, Department of Medicine, University of Colorado Anschutz Medical Campus. His compensation will be $5,000 for the total period or $2,500/year.

Mr. Rod Alne, US Air Force CMSgt Pararescue, Ret, a former Air Force pararescue specialist with extensive SOF and high altitude experience will serve as our special forces applications advisor. His compensation will be $5,000 for the total period or $2,500/year.
Total consultant cost for year 1 = 3* $2,500 = $7,500.
Total consultant cost for year 2 = 3* $2,500 = $7,500.

5. Subawards
We will be recruiting subjects living at sea level through collaboration with Dr. John Davis at Alma College in Michigan. Their costs are detailed in the sub award budget and budget justification. Depending on the duration of regulatory preparations, the sub award work might be happening mostly in year 1 with some overflow into year 2; for practical reasons the expenses have been fully included in year 1 of the budget. The direct cost of the sub award are $57,232 and the Indirect costs are $7,301.
Total subcontract cost year 1 = $64,533.

8. Subject Related Expenses
Assumption for subject-related expenses is that half of the subjects will have their altitude visit in year 1 and half in year 2. All totals therefore are split up equally over both years.

Transportation (70 subjects; Alma-Breckenridge): All subjects will fly to Denver from Grand Rapids, MI. Cost at this point are estimated at $400, hence $400/ticket x 70 = $28,000 for airfare. From Denver international Airport they will be transported to Breckenridge in mini-busses. Cost of the bus rental in Colorado is currently around $2,000 for the round-trip.
Total Transportation costs for year 1 = $20,000.
Total Transportation costs for year 2 = $20,000.

Lodging/meals: Based on our previous arrangements for a very successful field study conducted in the same location, we can house subjects for $75/pppd and feed them for $40 pppd. Cost for 70 subjects therefore is $17,500.
Total cost for lodging/meals year 1 = $8,750.
Total cost for lodging/meals year 2 = $8,750.

Field work staff consisting of 7 people will be needed for the altitude camp. Lodging, food, reimbursement for mileage are calculated at the GSA per diem rate of $143 for Breckenridge. Staff will have one preparatory trip prior to the first weekend. Cost for 7 people for one preparatory and 3 actual trips with 3 days in Breckenridge will amount to **$12,012 for year 1.** Three tips in **year 2 cost $9,009.**
Total subject related cost year 1 = $$20,000+$8,750+$12,012 = $40,762
Total subject related cost year 2 = $20,000+$8,750+$9,009 = $37,759

Office of Grants and Contracts
UNIVERSITY OF COLORADO DENVER | ANSCHUTZ MEDICAL CAMPUS

Fringe Benefit Rates

Office of Grants and Contracts

Treatment of Fringe Benefits

The University of Colorado Denver charges the actual cost of each fringe benefit direct to projects. However, it uses a fringe benefit rate which is applied to salaries and wages in budgeting fringe benefit costs under project proposals. The fringe benefits listed below are treated as direct costs.

UCD converted to using a fringe benefit rate for budgeting, effective for those applications/proposals submitted to outside sponsors on or after May 17, 1999. The change to a budgeting rate does not impact actual charges to funded projects as charges are based on actual costs.

The rates to be used for projects starting or extending beyond April 1, 2014 are as follows:

Project	Rate
Regular, Clinical, or Research Faculty & Professional Exempt *&**	28%
Post Doctorial Fellows & Other Faculty without regular appointment *&**	19%
Classified Staff	39%
Medical Residents (Contact GME) *** (<50%)	TBD
Part Time Faculty(<50%)	8%
Part Time Professional Research Assistant(<50%)	9%
Hourly Employees and Classified Temporary (Non Students)	18%
Students (Not Enrolled) ****	2%
Students (Enrolled) ****	1%

NOTE: The Fringe Rate allocations are now consistent across UCD therefore the above rates will be applicable to all UCD locations.

*Faculties with an appointment less than 100% of time but greater than or equal to 50% are eligible for health insurance at 100% of the University contribution rate. As a result benefits for these employees may be higher than the above rates.

** Faculty on contract pay do not accrue vacation and sick leave and are not subject to termination fringe for these employees reduce the given rate by 1%.

***GME is finalizing their costs in the interim please contact GME for assistance to use actual benefit amounts to calculate %.

**** To determine enrollment for students use the following:

- Undergraduate students enrolled in at least six credit hours during the academic year or at least three credit hours during the summer semester.
- Graduate students enrolled in at least five credit hours during the academic year or at least three credit hours during the summer semester.
- Not enrolled are those enrolled for less than above requirements

(http://www.ucdenver.edu/)

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 R&R

3. DATE RECEIVED BY STATE

State Application Identifier

1. TYPE OF SUBMISSION

❍Pre-application ●Application ❍Changed/Corrected Application

4. a. Federal Identifier

b. Agency Routing Identifier

c. Previous Grants.gov Tracking ID

2. DATE SUBMITTED

Applicant Identifier

5. APPLICANT INFORMATION **Organizational DUNS:** 800771594

Legal Name: The University of Texas Health Science Center at Houston

Department: Division:

Street1: P. O. Box 20036 Street2:

City: Houston County/Parish: Harris State: TX: Texas

Province: Country: USA: UNITED STATES ZIP / Postal Code: 77225-0036

Person to be contacted on matters involving this application

Prefix: First Name: Krystal Middle Name: Last Name: Toups Suffix:

Position/Title: Director, Grants

Street1: P.O. Box 20036 Street2:

City: Houston County/Parish: Harris State: TX: Texas

Province: Country: USA: UNITED STATES ZIP / Postal Code: 77225-0036

Phone Number: 713-500-3999 Fax Number: 713-383-3746 Email: preaward@uth.tmc.edu

6. EMPLOYER IDENTIFICATION NUMBER*(EIN) or (TIN)*: 741761309

7. TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍Women Owned ❍Socially and Economically Disadvantaged

8. TYPE OF APPLICATION:

●New ❍Resubmission

❍Renewal ❍Continuation ❍Revision

If Revision, mark appropriate box(es).

❍A. Increase Award ❍B. Decrease Award ❍C. Increase Duration

❍D. Decrease Duration ❍E. Other*(specify)*:

Is this application being submitted to other agencies?❍ Yes ● No What other Agencies?

9. NAME OF FEDERAL AGENCY:

Dept. of the Army -- USAMRAA

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

12.420

TITLE: Military Medical Research and Development

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Innovative metabolomic profiling reveals novel approaches to promote oxygen delivery in respiratory disease

12. PROPOSED PROJECT:

Start Date Ending Date

08/01/2015 07/31/2018

13. CONGRESSIONAL DISTRICT OF THE APPLICANT:

TX-009

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	First Name:	Middle Name:	Last Name:	Suffix:
Dr.	Yang		Xia	PhD

Position/Title: Professor
Organization Name: The University of Texas Health Science Center at Houston
Department: Biochem & Molecular Biology
Division: Medical School
Street1: 6431 Fannin St. MSB 6.200
Street2:
City: Houston
County/Parish: Harris
State: TX: Texas
Province:
Country: USA: UNITED STATES
ZIP / Postal Code: 77030-1501
Phone Number: 713-500-5039
Fax Number: 713-500-0652
Email: yang.xia@uth.tmc.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	$1,141,219.00
b. Total Non-Federal Funds	$0.00
c. Total Federal & Non-Federal Funds	$1,141,219.00
d. Estimated Program Income	$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree

The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL or other Explanatory Documentation. File Name: Mime Type:

19. Authorized Representative

Prefix:	First Name:	Middle Name:	Last Name:	Suffix:
	Krystal		Toups	

Position/Title: Director, Grants
Organization Name: The University of Texas Health Science Center at Houston
Department: Sponsored Projects
Division:
Street1: P.O. Box 20036
Street2:
City: Houston
County/Parish: Harris
State: TX: Texas
Province:
Country: USA: UNITED STATES
ZIP / Postal Code: 77225-0036
Phone Number: 713-500-3999
Fax Number: 713-383-3746
Email: preaward@uth.tmc.edu

Signature of Authorized Representative **Date Signed**

20. Pre-application File Name: Mime Type:

21. Cover Letter Attachment File Name: Mime Type:

Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: The University of Texas Health Science Center at Houston

* Street1: 6431 Fannin St.		Street2:
* City: Houston	County: Harris	* State: TX: Texas
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 77030-1501
DUNS Number: 800771594	* Project/Performance Site Congressional District: TX-009	

Project/Performance Site Location 1

Organization Name: University of Colorado Denver

* Street1: 12469 E. 17th Place		Street2:
* City: Denver	County:	* State: CO: Colorado
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 80217-3364
DUNS Number: 0410963140000	* Project/Performance Site Congressional District: CO-006	

Project/Performance Site Location 2

Organization Name: Alma College

* Street1: 614 West Superior Street		Street2:
* City: Alma	County:	* State: MI: Michigan
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 48801-1599
DUNS Number: 0725825050000	* Project/Performance Site Congressional District: MI-004	

	File Name	Mime Type
Additional Location(s)		

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Yang		Xia	PhD

Position/Title: Professor

Department: Biochem & Molecular Biology

Organization Name: The University of Texas Health Science Center at Houston

Division: Medical School

* Street1: 6431 Fannin St. MSB 6.200

Street2:

* City: Houston

County: Harris

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 77030-1501

*Phone Number	Fax Number	* E-Mail
713-500-5039	713-500-0652	yang.xia@uth.tmc.edu

Credential, e.g., agency login: yxia

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type:

Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Xia1015646988.pdf	application/pdf
Attach Current & Pending Support	Support_Xia1015647003.pdf	application/pdf

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Robert	C	Roach	

Position/Title: Associate Professor

Department: Emergency Medicine

Organization Name: University of Colorado Denver

Division: School of Medicine

* Street1: 12469 E. 17th Place, Box F524

Street2: Altitude Research Center

* City: Denver

County:

* State: CO: Colorado

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 80217-3364

*Phone Number	Fax Number	* E-Mail
303-724-1670		Robert.Roach@ucdenver.edu

Credential, e.g., agency login: rcroach

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: PhD

Degree Year: 1994

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Roach1015122336.pdf	application/pdf
Attach Current & Pending Support	Support_Roach1015646875.pdf	application/pdf

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	John	E	Davis	

Position/Title: Professor

Department: Integr Physiol & Health Sci

Organization Name: Alma College | Division:

* Street1: 614 W. Superior Street | Street2:

* City: Alma | County: | * State: MI: Michigan | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 48801-1504

*Phone Number	Fax Number	* E-Mail
989-463-7158		davisj@alma.edu

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Co-Investigator

Degree Type: PhD
Degree Year: 1985

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Davis1015647026.pdf	application/pdf
Attach Current & Pending Support	Support_Davis1015646874.pdf	application/pdf

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Rodney	E.	Kellems	PhD

Position/Title: Professor and Chairman | Department: Biochem & Molecular Biology

Organization Name: The University of Texas Health Science Center at Houston | Division: Medical School

* Street1: 6431 Fannin MSB 6.200 | Street2:

* City: Houston | County: Harris | * State: TX: Texas | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 77030-1501

*Phone Number	Fax Number	* E-Mail
713-500-6124	713-500-0652	rodney.e.kellems@uth.tmc.edu

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Collaborator

Degree Type: PhD
Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Kellems1015646907.pdf	application/pdf
Attach Current & Pending Support	Support_Kellems1015647004.pdf	application/pdf

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Andrew	W	Subudhi	

Position/Title: Associate Professor | Department: Emergency Medicine

Organization Name: University of Colorado Denver | Division: School of Medicine

* Street1: 12469 East 17th Place | Street2: Altitude Research Center

* City: Denver | County: | * State: CO: Colorado | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 80217-3364

*Phone Number	Fax Number	* E-Mail
303-724-1770		asubudhi@uccs.edu

Credential, e.g., agency login:		
*** Project Role:** Other (Specify)	**Other Project Role Category:** Co-Investigator	

Degree Type:
Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Subudhi1015647025.pdf	application/pdf
Attach Current & Pending Support	Support_Subudhi1015646876.pdf	application/pdf

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Yujin		Zhang	PhD

Position/Title: Assistant Professor

Department: Biochem & Molecular Biology

Organization Name: The University of Texas Health Science Center at Houston

Division: Medical School

* Street1: 6431 Fannin St. MSB 6.200

Street2:

* City: Houston

County: Harris

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 77030-1501

*Phone Number	Fax Number	* E-Mail
713-500-5981	713-500-0652	yujin.zhang@uth.tmc.edu

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Collaborator

Degree Type: PhD, Hematology
Degree Year: 1996

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Zhang1015646990.pdf	application/pdf
Attach Current & Pending Support	Support_Zhang1015647021.pdf	application/pdf

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Michael	R.	Blackburn	PhD

Position/Title: Professor and GSBS Dean

Department: Biochem & Molecular Biology

Organization Name: The University of Texas Health Science Center at Houston

Division: Medical School

* Street1: 6431 Fannin St. MSB 6.200

Street2:

* City: Houston

County: Harris

* State: TX: Texas

Province:

* Country: USA: UNITED STATES

* Zip / Postal Code: 77030-1501

*Phone Number	Fax Number	* E-Mail
713-500-6087	713-500-0652	Michael.R.Blackburn@uth.tmc.edu

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Other Significant Contributor

Degree Type:
Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Blackburn1015646871.pdf	application/pdf
Attach Current & Pending Support	Support_Blackburn1015647023.pdf	application/pdf

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Additional Senior/Key Person Form Attachments

When submitting senior/key persons in excess of 8 individuals, please attach additional senior/key person forms here. Each additional form attached here, will provide you with the ability to identify another 8 individuals, up to a maximum of 4 attachments (32 people).

The means to obtain a supplementary form is provided here on this form, by the button below. In order to extract, fill, and attach each additional form, simply follow these steps:

- Select the "Select to Extract the R&R Additional Senior/Key Person Form" button, which appears below.
- Save the file using a descriptive name, that will help you remember the content of the supplemental form that you are creating. When assigning a name to the file, please remember to give it the extension ".xfd" (for example, "My_Senior_Key.xfd"). If you do not name your file with the ".xfd" extension you will be unable to open it later, using your PureEdge viewer software.
- Using the "Open Form" tool on your PureEdge viewer, open the new form that you have just saved.
- Enter your additional Senior/Key Person information in this supplemental form. It is essentially the same as the Senior/Key person form that you see in the main body of your application.
- When you have completed entering information in the supplemental form, save it and close it.
- Return to this "Additional Senior/Key Person Form Attachments" page.
- Attach the saved supplemental form, that you just filled in, to one of the blocks provided on this "attachments" form.

Important: Please attach additional Senior/Key Person forms, using the blocks below. Please remember that the files you attach must be Senior/ Key Person Pure Edge forms, which were previously extracted using the process outlined above. Attaching any other type of file may result in the inability to submit your application to Grants.gov.

1) Please attach Attachment 1

2) Please attach Attachment 2

3) Please attach Attachment 3

4) Please attach Attachment 4

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)

Filename

MimeType

Additional Biographical Sketch(es) (Senior/Key Person)

Filename

MimeType

Additional Current and Pending Support(s)

Filename

MimeType

BIOGRAPHICAL SKETCH

Provide the following information for collaborators listed on this application. Follow this format for each person. **DO NOT EXCEED FOUR PAGES**

NAME:	POSITION TITLE:
Yang Xia	Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
Hunan Medical University, Changsha, China	M.D.	1986-1992	Medicine
Graduate School of Biomedical Science University of Texas at Houston, TX	Ph.D.	1993-1998	Molecular Pathology
University of Texas-Medical School, Houston, TX	Post Doc	1998-2001	Mouse Genetics

A. Personal Statement

I have a broad background in the cardiovascular field, with specific training and expertise in translational studies. My laboratory has used multidisciplinary approaches including genetic, pharmacological, cellular, biochemical and non-biased high throughput metabolomic screening to make *significant contribution in cardiovascular diseases.* Our goal is to translate our discovery-driven basic science smoothly and quickly to clinics to benefit human health. Our work is frequently published in prestigious journals including ***Nature Medicine, JCI, JEM, Circulation, Circulation Research, JASN, Hypertension and FASEB J***. Specifically, we have recently discovered that elevated adenosine signaling via A_{2B} adenosine receptor (ADORA2B) leads to O_2 release from erythrocytes by stimulating production of 2,3-diphosphoglycerate (2,3-DPG), an erythroid specific metabolite known to decrease hemoglobin (Hb) O_2 binding affinity (Zhang, et al, Nature Medicine, 2011). Recently, my laboratory has maintained a very productive and successful collaboration with Dr. Robert Roach who is an expert in human responses to hypoxia. With our combined efforts, we have generated strong preliminary studies from both human and animal supporting our overall hypothesis that increased circulating adenosine is likely beneficial to prevent hypoxia-induced pulmonary damage by enhance O_2 release. The research resulting from our collaborative efforts is likely to identify critical modulators and specific cell types involved in hypoxia-mediated adenosine response during the progression of disease and reveal innovative preventative and therapeutic possibilities for the disease at different stages. Finally, I am confident that our combined expertise along with the experience of our multidisciplinary team of collaborators places us in a favorable position to accomplish the goals of proposed research successfully and quickly.

B. Positions and Honors.

Professional Experience

2001-2004 Research Assistant Professor, Department of Biochemistry and Molecular Biology
The University of Texas Medical School at Houston

2004-2009 Assistant Professor, Department of Biochemistry and Molecular Biology
The University of Texas Medical School at Houston

2009-2013 Associate Professor, Department of Biochemistry and Molecular Biology
The University of Texas Medical School at Houston

2013-Present Professor, Department of Biochemistry and Molecular Biology
The University of Texas Medical School at Houston

Other Experience and Professional Memberships

2006-2010 Member, American Heart Association, Western Review Consortium Peer Review
2009 Member, NIHLB Special Study Section, Testing Mechanistic Hypotheses Generated by Findings from Genetic and Genomic Studies of Heart, Vascular, Lung, and Blood Disorders
2009 Member, NIHLB Special Study Section, Next Steps in Gene Discovery: Building upon Genome Wide Association Studies
2011-present Ad Hoc member, Pregnancy and Neonatology Study Section, National Institute of Health
2012-present NIHLB Advisory Committee on Sickle Cell Disease
2004-present Member, American Heart Association
1996-present Member, International Society for Heart Research
2013-present Member, American Society of Hematology

Honors

2014 Dean's Teaching Excellence Award, University of Texas Houston Medical School
2013 Dean's Teaching Excellence Award, University of Texas Houston Medical School
2012 Excellent Academic Achievement Award, XiangYa Oversea Alumni, USA
2011 Furong Scholar, China
2005 Winner, Outstanding Early Career Development Award, American Heart Association
2005 Finalist, Young Investigator Award, International Society for Heart Research
2004 Young Investigator Presentation Award, Placenta Association of American Conference
2003 AHA Texas Affiliate's Lyndon Baines Johnson Research Award

C. Selected Peer-reviewed Publications (most relevant publications in the recent 3 years and key publications from PI's laboratory)

1. Mi, T., Abbasi, S., Zhang, H., Uray, K., Chunn, J.L., Xia, L.W., Molina, J.G., Weisbrodt, N.W., Kellems, R.E., Blackburn, M.R. & **Xia, Y**. Excess adenosine in murine penile erectile tissues contributes to priapism via A2B adenosine receptor signaling. ***The Journal of clinical investigation*** 118, 1491-1501 (2008). PMID:18340377
2. Zhou, C.C., Zhang, Y., Irani, R.A., Zhang, H., Mi, T., Popek, E.J., Hicks, M.J., Ramin, S.M., Kellems, R.E. & **Xia, Y**. Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. ***Nature Medicine*** 14, 855-862 (2008). PMID:18660815.
3. Irani, R.A., Zhang, Y., Blackwell, S.C., Zhou, C.C., Ramin, S.M., Kellems, R.E. & **Xia, Y**. The detrimental role of angiotensin receptor agonistic autoantibodies in intrauterine growth restriction seen in preeclampsia. ***The Journal of experimental medicine*** *206,* 2809-2822 (2009). PMID:19887397
4. Wen, J., Jiang, X., Dai, Y., Zhang, Y., Tang, Y., Sun, H., Mi, T., Phatarpekar, P.V., Kellems, R.E., Blackburn, M.R. & **Xia, Y**. Increased adenosine contributes to penile fibrosis, a dangerous feature of priapism, via A2B adenosine receptor signaling. ***FASEB J*** 24:740-9. (2010). PMID:19858092
5. Dai, Y.B., Zhang, W.R., Zhang, Y.J., Wen, J.M., Kellems, R.E., and **Xia, Y.**: A2B adenosine receptor-mediated induction of IL-6 promotes CKD. ***Journal of American Society of Nephrology***, **22**: 890-901, 2011. PMID: 21511827
6. Zhang, Y.J., Dai, Y.B, Wen, J.M., Grenz, A., Sun, H., Tao, L.J., Lu, G.X., Danny, A.C., Milburn, M.V., Carter-Dawson, L., Lewis, D.E., Zhang, W.Z., Kellems, R.E., Eltzschig, H., Blackburn, M.R., Juneja, H.S., and **Xia, Y.**: Detrimental effects of adenosine signaling in sickle cell disease. ***Nature Medicine***, **17**: 79-86, 2011. PMID:21170046 (***Commentary by Nature Medicine and New Scientist***)
7. Wen, J.M., Dai, Y.B., Zhang, Y.J., Zhang, W.R., Kellems, R.E., Blackburn, M.R., Eltzschig, H.K., and **Xia, Y.**: Impaired erectile function in CD73-deficient mice with reduced intrinsic penile adenosine production. ***Journal of Sexual Medicine***, **8**(8):2172-80, 2011. PMID: 21595838

8. Wen, J.M., Grenz, A., Dai, Y.B., Zhang, Y.J, Kellems, R.E., Blackburn, M.R., Eltzschig, H.K., and **Xia, Y.**: A_{2B} adenosine receptor contributes to penile erection via PI3K/AKT signaling cascade-mediated eNOS activation. ***FASEB J***, **25**(8):2823-30, 2011. PMID: 21566208
9. Zhang, W.R., Yu, H., Zhang, Y.J., Dai, Y.B., Wang, W., Ning, C., Tao, L.J., Sun, H., Kellems, R.E., Blackburn, M.R., and **Xia, Y.**: Interleukin-6 underlies angiotension II-induced hypertension and chronic renal damage. ***Hypertension,*** **59**(1):136-44, 2012. PMID:22068875 (***Cover page)***
10. Karmouty-Quintana, H., Zhong, H., Acero, L., Weng, T., Melicoff-Portillo, E., West, J.D., Hemnes, A., Blackwell, T.S., **Xia, Y.**, Johnston, R.A., Zeng, D., Belardinelli, L., and Blackburn, M.R.: The adenosine A_{2B} receptor modulates pulmonary hypertension associated with interstitial lung disease. ***FASEB J*.,** 26(6):2546-57, 2012. PMID: 22415303
11. Grenz, A., Bauerle, J.D., Dalton, J.H., Ridyard, D., Badulak, A., Tak, E., McNamee, E., Clambey, E., Moldovan, R., Reyes, G., Klawitter, J., Ambler, K., Magee, K., Christians, U., Ravid, K., Choi, D.S., Wen, J., Lukashev, D., Blackburn, M.R., Osswald, H., Coe, I., Nürnberg, M., Haase, V.H., Finger, T., Xia, Y., Sitkovsky, M., and Eltzschig, H.K.: Selective role for equilibrative nucleoside transporter ENT1 in regulating renal reflow following acute kidney injury. ***Journal of Clinical Investigation***, 122(2):693-710, 2012. PMID:2269324
12. Ning, C., Wen, J.M., Qi, L., Zhang, Y.J., Zhang, W.R., Wang, W., Blackburn, M.R., Kellems, R.E., and **Xia, Y.**: Excessive penile norepinephrine levels underlies impaired penile erection in adenosine A1 receptor deficient mice. ***Journal of Sexal Medicine***, **9**(10):2552-61, 2012. PMID:22862844
13. Wang, W., Irani, R.A., Blackwell, S.C., Ramin, S.M., Tao, L.J., Kellems, R.E., and **Xia, Y.**: Autoantibody-induced complement activation contributes to pathophysiology in a model of preeclampsia. ***Hypertension***, **60**(3):712-21, 2012. PMID:22868393
14. Zhang, W.R., Yu, H., Dai, Y.B., Zhang, Y.J., Wang, W., Ning, C., Luo, R., Sun, K., Grenz, A., Sun, H., Tao, L., Zhang, W., Colgan, S., Blackburn, M.R., Etlzschig, H.K., Kellems, R.E., and **Xia, Y.**: Elevated renal adenosine is a detrimental mediator underlying hypertension and disease progression. ***Circulation Research***, 112(11):1466-78, 2013. PMID: 23603835
15. Eckle, T., Hughes, K., Ehrentraut, H., Brodsky, K.S., Rosenberger, P., Choi, D.S., Ravid, K., Weng, T., **Xia,** Y., Blackburn, M.R., Eltzschig. H.K.: Crosstalk between the equilibrative nucleoside transporter ENT2 and alveolar Adora2b adenosine receptors dampens acute lung injury. ***FASEB J***., 27(8):3078-89, 2013. PMID:23603835
16. Karmouty-Quintana, H., Weng, T.T., Garcia-Morales, L.J., Chen, L.Y., Pedroza, M., Zhong, H.Y., Molina, J.D., Bunge. R., Bruckner, B.R., Xia, Y., Johnston, R.A., Loebe, M., Zeng, D., Seethamraju, H., Belardinelli, L., & Blackburn, M. : ADORA2B and Hyaluronan modulate Pulmonary Hypertension secondary to Chronic Obstructive Pulmonary Disease. ***Am J Respir Cell Mol Biol***. 49(6):1038-47, 2013. PMID:23855769
17. Wen, J. and **Xia, Y**.: Adenosine signaling, good or bad in erectile function. ***Atherosclerosis Thrombosis Vascular Biology***, 32(4):845-50 (2012) PMID:22423035
18. Zhang, Y.J. and Xia, Y.: Adenosine signaling in normal and sickle erythrocytes and beyond. ***Microbes and Infection,*** 14(10):863-73, 2012. PMID:22634345
19. Sun, K. and **Xia, Y**.: New insights into Sickle Cell Disease: A disease of hypoxia. ***Current Opinion of Hematology***, 20(3):215-21, 2013. PMID:23549375
20. Karmouty-Quintana H, **Xia Y**, Blackburn MR: Adenosine signaling during acute and chronic lung disease, ***J Mol Med (Berl***), 91(2):173-81, 2013. PMID:23340998
21. Liu, C., Wang, W., Parchim, N., Irani, R., Saibi, B., Blackwell, S.C., Jin, J.P., Kellems, R.E. and **Xia, Y**.: Tissue transglutaminase contributes to the pathogenesis of preeclampsia and stabilizes placental angiotensin receptor AT1 by ubiquitination-preventing isopeptide modification. ***Hypertension***, 63(2):353-61, 2013. PMID:24191290
22. Wang, W., Parchim, N., Liu, C., Irani, R.A., Zhang, W.R., Chen, N., Zhang, Y.J., Blackwell, S.C., Tao, L.J., Kellems, R.E., and **Xia, Y.**: Excess LIGHT contributes to placental impairment, toxic factor secretion,

hypertension and proteinuria in preeclampsia by activating its receptors. ***Hypertension***, *63(3):595-606.* 2014, *PMID:24324043*

23. Elliott, S.E., Parchim, N.F., Liu, C., **Xia,** Y., **Kellems,** R.E., Soffici, A.R., Daugherty, P.S. : Characterization of antibody specificities associated with preeclampsia, ***Hypertension,*** 63(5):1086-93, 2014, PMID:24446060
24. Chen, N., Wen, J.M., Dai, Y.B., Zhang, Y.J., Kellems, R.E., and **Xia, Y.**: Excess adenosine A2B receptor signaling contributes to priapism through HIF-1α mediated reduction of PDE5 gene expression. ***FASEB J.*** *28(6):2725-2735****,*** 2014, PMID:24614760
25. Li, C., Peart, N., Xuan, Z., **Lewis,** D.E., **Xia, Y**., Jin, J.: PMA induces SnoN proteolysis and CD61 expression through an autocrine mechanism, ***Cell Signal***. 26(7):1369-1378, 2014, PMID: 24637302
26. Zhang, Y.J., Berka,V., Song, A.R., Sun, K.Q., Wang, W., Zhang, W.R., Ning, C., Li, C.H., Zhang, Q.B.,., Alexander, D.C., Milburn, M.V., Ahmed, M.H., Idowu, I., Zhang, J., Kato, G., Abdulmalik, O.Y., Zhang, W.Z., Bogdanov, M, Dowhan, W., Kellems, R.E., Safo, M., Tsai, A.T., Juneja H.S., and **Xia, Y.**: Elevated sphingosine-1-phosphate promotes sickling and sickle cell disease progression, ***Journal of Clinical Investigation****,* , 124(7): 2750-61, 2014, PMCID: PMC4071396

D. Research Support (Most Recent Five years)

Active Research Support

1R01HL119549 (PI Yang Xia) 02/01/13-01/31/17 NIH NHLBI
"Metabolites, sickle cell disease and novel therapeutics"
To determine newly identified metabolites in sickle cell disease and develop new therapies for the disease.

P01HL114457-01 (PD (Michael Blackburn) 06/01/13-5/31/18 NIH NHLBI
Project 3: "**Novel role of erythrocyte in hypoxia adenosine response**" (PI Yang Xia)
The goal is to assess the role of erythrocyte function in hypoxia-mediated elevation of adenosine in tissue injury.

Complete Research Support

1R01DK083559-01 (PI Yang Xia) 05/01/09-03/31/14 NIH NIDDK
"Adenosine Signaling, Priapism and Sickle Cell Disease"
The goal is to determine the molecular mechanisms for adenosine-induced priapism in sickle cell disease.

12IRG9150001 (PI Yang Xia) 01/01/12-12/31/13 AHA
"Sickle Cell Anemia, Vascular Endothelial Dysfunction and Novel Therapeutics"
The goal of the proposed research is the development of novel approaches to ameliorate vascular endothelial dysfunction and prevent multiple life-threatening complications associated with sickle cell anemia including pulmonary hypertension (PH) and stroke.

1RC4HD067977-01 (PI Yang Xia and RE Kellems) 09/30/10-09/30/13 NIH NICHD
"Autoantibodies in Preeclampsia: Pre-symptomatic markers and therapeutic targets"
To determine if autoantibodies serve as pre-symptomatic markers and therapeutic targets in preeclampsia.

2R01HD034130 (PI RE Kellems, Co-PI) 02/15/08-01/31/13 NIH NICHD
"Preeclampsia and Autoimmunity"
The goal is to determine how angiotensin receptor agonistic autoantibodies cause preeclampsia.

10GRNT3760081 (PI Yang Xia) 07/01/10-06/30/12 AHA
"**Autoantibody-Induced Inflammatory Response Underlies the Pathogenesis of Preeclampsia"**
To determine whether increased inflammatory response underlies autoantibody-induced preeclampsia.

Biographical Sketch

Provide the following information for each individual included in the Research & Related Senior/Key Person Profile (Expanded) Form.			
NAME **ROBERT C. ROACH, PH.D.**		POSITION TITLE **ASSOCIATE PROFESSOR**	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).			
INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
The Evergreen State College, Olympia, WA	B.S.	1979	Biochemistry
Cornell University, Ithaca, NY	M.S.	1985	Nutritional Science
University of New Mexico, Albuquerque, NM	Ph.D.	1994	Exercise Physiology

RESEARCH AND PROFESSIONAL EXPERIENCE:

1989-2005 Associate Scientist, Siberian-Alaskan Medical Research Exchange, Section Cold Altitude Physiology, University of Alaska, Anchorage, AK
1990-1994 Research Physiologist, Oxygen Transport Program, The Lovelace Institutes, Albuquerque, NM
1993-2005 Consultant, Life Support Systems, Odyssey Around the World Balloon Flight, Albuquerque, NM
1994-1996 Associate Scientist, Cardiopulmonary Physiology, Institute Basic Applied Medical Research, The Lovelace Institutes, Albuquerque, NM
1996-1998 Alfred Benzon Research Fellow, Copenhagen Muscle Research Center, Copenhagen, Denmark.
1998-1999 Visiting Professor, Department of Life Sciences, New Mexico Highlands University, Las Vegas, NM
1999-2000 Research Assistant Professor, Department Life Sciences, New Mexico Highlands University, Las Vegas, NM
1999-2003 Clinical Assistant Professor, Department Medicine, University of New Mexico, Albuquerque, NM
1999-2002 Clinical Assistant Professor, Department Surgery, Div Emergency Medicine, UCHSC
1999-present Co-Chairman, International Hypoxia Symposia (www.hypoxia.net)
2001-2003 Scientist, New Mexico Resonance, Albuquerque, NM
2003-2010 Associate Director and Chief, Research Division, Altitude Research Center UCHSC, Denver, CO
2010-present Director, Altitude Research Center, University of Colorado Denver, Denver, CO

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INDIVIDUAL.

PROFESSIONAL MEMBERSHIPS

American Physiological Society; American College of Sports Medicine; American Association for the Advancement of Science; American Alpine Club; International Society for Mountain Medicine

REVIEW AND REFEREE WORK

Appointed, Editorial Board, Journal of Applied Physiology, 2006 to present
Appointed, Editorial Board, High Altitude Medicine and Biology, 2006 to present
Appointed, Editorial Board, Medicine Science Sports and Exercise, 2005 to 2011
Appointed, Section Editor, Hypoxia, Extreme Medicine and Physiology, BMC Journals, 2011-present.
Invited Reviewer, DOD Brain Injury Study Section, American Institute of Biological Science, 2009-2010.

HONORS AND AWARDS

Elected Fellow, American College of Sports Medicine (FACSM), fall 2004.
Appointed, American Physiological Society Porter Scholarship Selection Committee, 2005-2008
Appointed, American College of Sports Medicine, Constitution, Bylaws and Operating Codes Committee, 2006-2009
Appointed, American College of Sports Medicine, Promotions and Fellowship Committee, 2011 to present

PUBLICATIONS (from 115 total publications, 19 in last three years)

1. Asgari S, Subudhi AW, Roach RC, Liebeskind DS, Bergsneider M, Hu X. An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. Journal of neuroscience methods 2011;197:171-9.
2. Browne VA, Toledo-Jaldin L, Davila RD, et al. High-end arteriolar resistance limits uterine artery blood flow and restricts fetal growth in preeclampsia and gestational hypertension at high altitude. American journal of physiology Regulatory, integrative and comparative physiology 2011;300:R1221-9.
3. Julian CG, Subudhi AW, Wilson MJ, Dimmen AC, Pecha T, Roach RC. Acute mountain sickness, inflammation, and permeability: new insights from a blood biomarker study. Journal of Applied Physiology 2011;111:392-9.
4. Roach R, Hackett P, Kayser B. Pro: Rebuttal. High Altitude Medicine & Biology 2011;12:27-.
5. Roach R, Kayser B, Hackett P. Pro: Headache should be a required symptom for the diagnosis of acute mountain sickness. High altitude medicine & biology 2011;12:21-2; discussion 9.
6. Subudhi AW, Dimmen AC, Julian CG, Wilson MJ, Panerai RB, Roach RC. Effects of acetazolamide and dexamethasone on cerebral hemodynamics in hypoxia. Journal of applied physiology 2011;110:1219-25.
7. Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B, Roach RC. Does cerebral oxygen delivery limit incremental exercise performance? Journal of applied physiology 2011;111:1727-34.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INDIVIDUAL.

8. Wilson MJ, Julian CG, Roach RC. Genomic analysis of high altitude adaptation: innovations and implications. Current sports medicine reports 2011;10:59-61.
9. Ezzati M, Horwitz ME, Thomas DS, et al. Altitude, life expectancy and mortality from ischaemic heart disease, stroke, COPD and cancers: national population-based analysis of US counties. J Epidemiol Community Health 2012;66:e17.
10. Olin JT, Dimmen AC, Subudhi AW, Roach RC. A simple method to clamp end-tidal carbon dioxide during rest and exercise. Eur J Appl Physiol 2012;112:3439-44.
11. Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC. AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans. Journal of applied physiology 2013;115:634-42.
12. Fan JL, Subudhi AW, Evero O, et al. AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure. Journal of applied physiology 2014;116:911-8.
13. Goodall S, Twomey R, Amann M, et al. AltitudeOmics: exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude. Acta physiologica 2014;210:875-88.
14. Julian CG, Subudhi AW, Hill RC, et al. Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness in humans. Journal of applied physiology 2014;116:937-44.
15. Roach EB, Bleiberg J, Lathan CE, Wolpert L, Tsao JW, Roach RC. AltitudeOmics: Decreased reaction time after high altitude cognitive testing is a sensitive metric of hypoxic impairment. Neuroreport 2014.
16. Roach RC, Wagner PD, Hackett PH. Translation in progress: hypoxia. Journal of applied physiology 2014;116:835-6.
17. Subudhi AW, Bourdillon N, Bucher J, et al. AltitudeOmics: the integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent. PLoS One 2014;9:e92191.
18. Subudhi AW, Fan JL, Evero O, et al. AltitudeOmics: cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. Journal of applied physiology 2014;116:724-9.
19. 19. Subudhi AW, Fan JL, Evero O, et al. AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. Experimental physiology 2014.

Biographical Sketch

Provide the following information for each individual included in the Research & Related Senior/Key Person Profile (Expanded) Form.			
NAME **JOHN E. DAVIS**		POSITION TITLE **CHARLES A. DANA PROFESSOR OF INTEGRATIVE PHYSIOLOGY**	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).			
INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Kenyon College, Gambier, Ohio	B.A.	1971-75	Biology
State University College at Buffalo, NY	M.S.	1976-78	Biology
State University College at Buffalo, NY	Ph.D.	1981-84	Exercise Science
The John Hopkins University, Baltimore, Maryland	Post Doctoral fellow	1984-85	Environmental Physiology

RESEARCH AND PROFESSIONAL EXPERIENCE:

PROFESSIONAL EXPERIENCE

1985-1991 Assistant Professor, Department of Exercise and Health Science, Alma College, Alma, Michigan.

1991-1997 Associate Professor and Department Chair, Department of Exercise and Health Science, Alma College, Alma, Michigan.

1999-2000 Visiting Professor, Department of Bioscience, University of Hertfordshire, Hatfield, United Kingdom

1997-2003 Professor and Department Chair, Department of Exercise and Health Science, Alma College, Alma, Michigan.

2013-2104 Visiting Professor, Department of Movement Sciences, Utah State Unviersity, Logan, Utah

2003-present Charles A. Dana Professor of Integrative Physiology and Health Science. Alma College, Alma, Michigan

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INDIVIDUAL.

HONORS

Victor M. Hawthorne New Investigator Award- 1988
Alma College Barlow Award for Faculty Excellence - 1990, 1997
Nominated by Alma College for Carnegie Foundation Professor of the year award - 1996, 1997,1999
Posey Award for Research and Teaching - 2000
Awarded Charles A. Dana Endowed Professorship – 2003

ADVISORY COMMITTEES:

National Science Foundation STEP (Science Talent Expansion Program) Advisory Committee 2012 - 2014

PUBLICATIONS (Relevant publications including published abstracts)

1. Fortney, S.M., K.H. Hyatt, J.E. Davis, and J.M. Vogel. Changes in Body Fluid Compartments During a 28-Day Bed Rest. Aerospace Medicine: 62: 97-104, 1991.
2. Frey, M.A., C. Lathers, J. E. Davis, S. Fortney, and J.B. Charles. Cardiovascular responses to standing: Effect of hydration. J. Clin. Pharmacol. 34:387-393, 1994.
3. Davis, J.E., W.R. Pourcho, and C.M. Thomas. Effect of maximal exercise on autonomic control of heart rate during orthostatic stress. In Environmental Ergonomics:Recent Progress and New Frontiers Editors: Y. Shapiro, D.S. Moran, and Y. Epstein. Freund Pub. House. London, 1996. p. 381-385.
4. Davis, J.E. and S.M. Fortney. Effect of Fluid Ingestion on Orthostatic Responses Following Acute Exercise. International Journal of Sports Medicine 18(3):174-178, 1997.
5. Davis, J.E., K.E. Horwood, and G.K. DeJong. Effects of Exercise During Simulated Microgravity on Postural Control. Aviation, Space, and Environmental Medicine 68:392-395, 1997.
6. Ventline, H.L., L.M. Girodano, M.J. Luetkemeier, and J.E. Davis. Effects of Moderate Altitude on Peripheral Sweating. Medicine and Science and Sports and Exercise 36(5): S109, 2004.
7. Hawkins, K., B. hauser, J.E. Davis, and M.J. Luetkemeier. Effect of Altitude on Lactate Removal Rates Following High-Intensity Exercise. Medicine and Science and Sports and Exercise 36(5): S109, 2004.
8. Montoye, A. , C. McCue, N. Garvin, B. Gervasi, and J. E. Davis.Effect of Moderate Altitude on EPOC following Maximal Exercise. Medicine and Science and Sports and Exercise 41(5): S228, 2009.
9. L. J. Hasler, D. Johnson, M. J. Luetkemeier, A. Carlson, Katy J. Green, and J. E. Davis. Effects of exercise at moderate altitude on sweat composition and sweat rates. Medicine and Science and Sports and Exercise 41(5): S371, 2009.
10. Davis, J.E., S. A. Swanton, G.L. Gaskell, and K. Walsh. Effect of Moderate Altitude on Lactate Clearance During Active and Passive Recovery. Medicine and Science and Sports and Exercise 41(5):S568, 2009.

11. Hrutkay, S., J. Thorington, S. Lux and J.E. Davis. Effects of Acute and Chronic Exposure to Altitude onForearm Blood Flow and Reactive Hypermia. Medicine Science and Sports in Exercise 43(5): S446,2011.
12. Grant, A, M. Perez, and J.E. Davis. Effect of Altitude of Residence on the Cardiovascular Responses to Dynamic and Isometric Hand-Grip. Medicine Science and Sports in Exercise 44(5): S667, 2012.
13. Gooding, B., J. Cole, , D. Hicks, M. Clark, and J.E. Davis. Effects of Moderate Altitude on Oxygen Debt, Oxygen Deficit, and the Onset of Blood Lactate during Exercise. Medicine Science and Sports in Exercise 45(5): S351, 2013.
14. Jongerkrijg, L., S. Clancy, J. K. Murawske, and J. E. Davis. Effects of Aerobic and Anaerobic Training on Aerobic Capacity and Blood Hematology at 3400 meters. Medicine Science and Sports in Exercise 45(5): S486, 2013.
15. Davis , J.E., D.R. Wagner, J. Thorington,and C. Schall. Orthostatic Responses at 4860 m in Low, Moderate, and High Altitude Residents. High Altitude Medicine and Biology 14(3): 251-254, 2013.
16. Wagner, D.R., J. E Davis, T. Payne, and W. Hussain. Muscle Oxygenation during Dynamic and Isometric Exercise in High Altitude-Resident Guides, Climbers and Tourists at 4810 m. High Altitude Medicine and Biology 15 (2): A-273, 2014.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kellems, Rodney E.	Professor & Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bellarmine University, Louisville, KY	A.B.	1969	Biology
Princeton University, Princeton, N.J.	Ph.D.	1974	Biochemistry
Stanford University, Stanford, CA	Postdoctoral	1974-1978	Molecular Genetics

A. Personal Statement: I have maintained an active research interest in adenosine deaminase (ADA) deficiency for over thirty years. To obtain the biochemical, immunologic and genetic reagents needed for our research my laboratory initially devised selection protocols to isolate mammalian cell lines with highly amplified copies of murine ADA genes. Using these cell lines we were the first to obtain purified enzyme, monospecific antibody, cDNA and genomic clones. We produced ADA-deficient mice using a combined genetic strategy of targeted gene disruption followed by transgenic based placental specific expression of ADA to achieve rescue from perinatal lethality. The resulting ADA-deficient mice have been successfully used by us and others to determine the metabolic and molecular basis of the immune phenotype characteristic of ADA deficiency in humans and as pre-clinical models to test the feasibility of ADA gene therapy in humans. Because ADA-deficient mice are characterized by chronically elevated adenosine, they have served as a valuable experimental model to identify tissues and organs where excessive adenosine signaling creates a pathological phenotype (e.g. lungs, liver, penis, bones, placenta). Although these discoveries are based on pathological phenotypes resulting from excessive adenosine signaling, they have served to identify organs where properly regulated adenosine signaling plays a normal physiological role.

Dr. Xia provides substantial expertise in adenosine signaling, mouse genetics and translational studies. Her laboratory has produced or acquired mice with genetic deficiencies in all relevant aspects of adenosine signaling. Together, we are well poised to bring the proposed research to a successful conclusion. Our track record of 47 co-authored publications provides tangible evidence of our productive working relationship.

B. Positions and Honors

Professional Experience

1978 – 1983	Assistant Professor, Department of Biochemistry, Baylor College of Medicine
1983 – 1988	Associate Professor, Department of Biochemistry, Baylor College of Medicine
1985 – 1988	Associate Professor, Dept of Molecular & Human Genetics, Baylor College of Medicine
1988 – 1997	Professor, Department of Molecular and Human Genetics, Baylor College of Medicine
1988 – 1997	Professor, Department of Biochemistry, Baylor College of Medicine
1997 – Present	Professor & Chairman, Medical School, Dept of Biochemistry & Molecular Biology, UTHSC-H

Honors and Awards

1965 – 1969	President's Scholarship, Bellarmine College
1969 – 1971	National Institutes of Health, Predoctoral Traineeship, Princeton University
1974 – 1976	National Institutes of Health, Postdoctoral Fellowship, Stanford University
1982 – 1987	U.S. PHS, Research Career Development Award, Baylor College of Medicine
1988 – 1993	Member, Mammalian Genetics Study Section
1993 – 1997	Member, NIH Reviewer's Reserve

Professional Organizations:
American Association for the Advancement of Science
American Society for Biochemistry and Molecular Biology
American Association of Immunologists

C. Selected Peer-reviewed Publications (from over 129)

1. Zhang Y, Dai Y, Wen J, Zhang W, Grenz A, Sun H, Tao L, Lu G, Alexander DC, Milburn MV, Carter-Dawson L, Lewis DE, Zhang W, Eltzschig HK, **Kellems RE**, Blackburn MR, Juneja HS, Xia Y. Detrimental effects of adenosine signaling in sickle cell disease. Nat Med 17(1):79-86, 2011.
2. Siddiqui, A.H., Irani, R.A., Zhang, Y., Dai, Y., Blackwell, S.C., Ramin, S.M., **Kellems, R.E.**, Xia, Y.: Recombinant vascular endothelial growth factor 121 attenuates autoantibody-induced features of preeclampsia in pregnant mice. *Am. J. Hypertens.,* **24**(5):606-612, 2011. PMCID: PMC3262171
3. Zhou, C.C., Irani, R.A., Dai, Y., Blackwell, S.C., Hicks, M.J., Ramin, S.M., **Kellems, R.E.**, Xia, Y.: Autoantibody-mediated IL-6-dependent endothelin-1 elevation underlies pathogenesis in a mouse model of preeclampsia. *J. Immunol.,* **186**(10):6024-6034, 2011. PMCID: PMC3269191
4. Dai Y, Zhang W, Wen J, Zhang Y, **Kellems RE**, Xia Y. A2B adenosine receptor-mediated induction of IL-6 promotes CKD. *J Am Soc Nephrol* **22**(5):890-901, 2011. PMCID: PMC3083311
5. Wen J, Grenz A, Zhang Y, Dai Y, **Kellems RE**, Blackburn MR, Eltzschig HK, Xia Y. A2B adenosine receptor contributes to penile erection via PI3K/AKT signaling cascade-mediated eNOS activation. *FASEB J* **25**(8):2833-30, 2011. PMCID: PMC3136334
6. Wen J, Dai Y, Zhang Y, Zhang W, **Kellems RE**, Xia Y. 13. Impaired erectile function in CD73-deficient mice with reduced endogenous penile adenosine production. *J Sex Med* **8**(8):2172-80, 2011. PMCID: PMC3380606
7. Xia, Y., **Kellems, R.E.**: Receptor-activating autoantibodies and disease: preeclampsia and beyond. *Expert Rev. Clin. Immunol.,* **7**(5):659-674, 2011. PMCID: PMC3268148
8. Zhang, W., Wang, W., Yu, H., Zhang, Y., Dai, Y., Ning, C., Tao, L., Sun, H., **Kellems, R.E.**, Blackburn, M.R., Xia, Y.: Interleukin 6 underlies angiotensin II-induced hypertension and chronic renal damage. *Hypertension,* **59**(1):136-144, 2012. PMCID: PMC3842011
9. Zhou, C.C., Chang, J., Mi, T., Abbasi, S., Gu, D., Huang, L., Zhang, W., **Kellems, R.E.**, Schwartz, R.J., Xia, Y.: Targeted expression of Cre recombinase provokes placental-specific DNA recombination in transgenic mice. *PLoS One,* **7**(2):e29236, 2012. PMCID: PMC3281813
10. Wang, W., Irani, R.A., Zhang, Y., Ramin, S.M., Blackwell, S.C., Tao, L., **Kellems, R.E.**, Xia, Y.: Autoantibody-mediated complement C3a receptor activation contributes to the pathogenesis of preeclampsia. *Hypertension,* **60**(3):712-721, 2012. PMID: 22868393
11. Siddiqui, A.H., Irani, R.A., Zhang, W., Wang, W., Blackwell, S.C., **Kellems, R.E.**, Xia, Y.: Angiotensin receptor agonistic autoantibody-mediated soluble fms-like tyrosine kinase-1 induction contributes to impaired adrenal vasculature and decreased aldosterone production in preeclampsia. *Hypertension,* **61**(2):472-479, 2013. PMID: 23283357
12. Zhang W, Zhang Y, Wang W, Dai Y, Ning C, Luo R, Sun K, Glover L, Grenz A, Sun H, Tao L, Zhang W, Colgan SP, Blackburn MR, Eltzschig HK, **Kellems RE**, Xia Y. Elevated ecto-5'-nucleotidase-mediated increased renal adenosine signaling via A2B adenosine receptor contributes to chronic hypertension. *Circ Res* **112**(11):1466-78, 2013. PMCID: PMC3886128
13. Xia, Y, **Kellems, R.E.**: Angiotensin receptor agonistic autoantibodies and hypertension: preeclampsia and beyond. *Circ Res,* **113**(1):78-87, 2013.
14. Wang, W., Parchim, N., Iriyama, T., Luo, R., Zhao, C., Liu, C., Irani, R.A., Zhang, W., Ning, C., Zhang, Y., Blackwell, S.C., Chen, L., Tao, L., Hicks, J., **Kellems, R.E.**, Xia, Y.: Excess LIGHT contributes to placental impairment, increased secretion of vasoactive factors, hypertension and proteinuria in preeclampsia. *Hypertension* [Epub ahead of print], 2013. PMCID: PMC4053533
15. Liu, C., Wang, W., Parchim, N., Irani, R.A., Blackwell, S., Sibai, B., Jin, J., **Kellems, R.E.**, Xia, Y.: Tissue transglutaminase contributes to the pathogenesis of preeclampsia and stabilizes placental

angiotensin II receptor type 1 by ubiquitination-preventing isopeptide modification. *Hypertension* **63**(2):353-61, 2014. PMCID: PMC4052572

16. 15 Elliott ,S.E., Parchim, N.F., Liu, C., Xia, Y., **Kellems, R.E.**, Soffici, A.R., Daugherty, P.S.: Characterization of Antibody Specificities Associated With Preeclampsia. *Hypertension* [Epub ahead of print], 2014. PMCID: PMC3984369
17. Ning C, Wen J, Zhang Y, Dai Y, Wang W, Zhang W, Qi L, Grenz A, Eltzschig HK, Blackburn MR, **Kellems RE**, Xia Y. 2. Excess adenosine A2B receptor signaling contributes to priapism through HIF-1α mediated reduction of PDE5 gene expression. *FASEB J* **28**(6):2725-35, 2014. PMCID: PMC4021439
18. Zhang Y, Berka V, Song A, Sun K, Wang W, Zhang W, Ning C, Li C, Zhang Q, Bogdanov M, Alexander DC, Milburn MV, Ahmed MH, Lin H, Idowu M, Zhang J, Kato GJ, Abdulmalik OY, Zhang W, Dowhan W, **Kellems RE**, Zhang P, Jin J, Safo M, Tsai AL, Juneja HS, Xia Y. 1. Elevated sphingosine-1-phosphate promotes sickling and sickle cell disease progression. *J Clin Invest* **124**(6):2570-61, 2014. PMCID in process.

Additional earlier publications relevant to this application

Carbonaro, D.A., Jin, X., Cotoi, D., Mi, T., Yu, X.J., Skelton, D.C., Dorey, F., **Kellems, R.E.**, Blackburn, M.R., Kohn, D.B.: Neonatal bone marrow transplantation of ADA-deficient SCID mice results in immunological reconstitution despite low levels of engraftment and an absence of selective donor T lymphoid expansion. *Blood,* **111**(12):5745-5754, 2008. [PMID: 18356486]

Mi, T., Abbasi, S., Zhang, H., Uray, K., Chunn, J.L., Xia, L.W., Molina, J.G., Weisbrodt, N.W., **Kellems, R.E.**, Blackburn, M.R., Xia, Y.: Excess adenosine in murine penile erectile tissues contributes to priapism via A(2B) adenosine receptor signaling. *J Clin Invest*., **118**(4):1491-1501, 2008. [PMID: 18340377]

Carbonaro, D.A., Jin, X., Petersen, D., Wang, X., Dorey, F., Kil, K.S., Aldrich, M., Blackburn, M.R., **Kellems, R.E.**, Kohn, D.: In vivo transduction by intravenous injection of a lentiviral vector expressing human ADA into neonatal ADA gene knock-out mice: A novel form of enzyme replacement therapy for ADA-deficiency. *Molecular Therapy,* **13**(6):1110-1120, 2006. [PMID: 16651028]

Chunn, J.L., Mohsenin, A., Young, H.W., Lee, C.G., Elias, J.A., **Kellems, R.E.**, Blackburn, M.R.: Partially adenosine deaminase-deficient mice develop pulmonary fibrosis in association with adenosine elevations. *Am J Physiol Lung Cell Mol Physiol* **290**(3):L579-587, 2006. [PMID: 16258000]

Chunn, J.L., Molina, J.G., Mi, T., Xia, Y., **Kellems, R.E.**, Blackburn, M.R.: Adenosine-dependent pulmonary fibrosis in adenosine deaminase-deficient mice. *J Immunol,* **175**(3):1937-1946, 2005. [PMID: 16034138]

Blackburn, M.R., **Kellems, R.E.**: Adenosine deaminase deficiency: Metabolic basis of immune deficiency and pulmonary inflammation. *Adv Immunol*, **86**:1-41, 2005. [PMID: 15705418]

Aldrich, M.B., Chen, W., Blackburn, M.R., Martinez-Valdex, H., Datta, S.K., **Kellems, R.E.**: Impaired germinal center maturation in adenosine deaminase deficiency. *J Immunol*, **171**(10):5562-5570, 2003. [PMID: 14607964]

Apasov, S.G., Blackburn, M.R., **Kellems, R.E.**, Smith, P.T., Sitkovsky, M.V.: Adenosine deaminase deficiency increases thymic apoptosis and causes defective T cell receptor signaling. *J Clin Invest,* **108**(1):131-141, 2001. [PMID: 11435465]

Thompson, L.F., Van De Wiele, C.J., Laurent, A.B., Hooker, S.W., Vaughn, J.G., Jiang, H., Khare, K., **Kellems, R.E.**, Blackburn, M.R., Hershfield, M.S., Resta, R.: Metabolites from apoptotic thymocytes inhibit thymopoiesis in adenosine deaminase-deficient fetal thymic organ cultures. *J Clin Invest,* **106**(9)**:**1149-57, 2000. [PMID: 11067867]

Blackburn, M.R., Aldrich, M., Volmer, J.B., Chen, W., Zhong, H., Kelly, S., Hershfield, M.S., Datta, S.K., **Kellems, R.E.**: The use of enzyme therapy to regulate the metabolic and phenotypic consequences of adenosine deaminase deficiency in mice: Differential impact on pulmonary and immunologic abnormalities. *J Biol Chem,* **275**(41)**:**32114-32121, 2000. [PMID: 10908569]

Blackburn, M.R., Volmer, J.B., Thrasher, J.L., Zhong, H., Crosby, J.R., Lee, J.J., **Kellems, R.E.**: Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation, and airway obstruction. *J Exp Med*, **192**(2):159-170, 2000. [PMID: 10899903]

Blackburn, M.R., Datta, S.K., **Kellems, R.E.**: Adenosine deaminase-deficient mice generated using a two-stage genetic engineering strategy exhibit a combined immunodeficiency. *J Biol Chem,* **273**(9):5093-5100, 1998. [PMID: 9478961]

Blackburn, M.R., Knudsen, T.B., **Kellems, R.E.**: Genetically engineered mice demonstrate that adenosine deaminase is essential for early postimplantation development. *Development,* **124**(16):3089-3097, 1997. [PMID: 9272950]

Blackburn, M.R., Datta, S.K., Wakamiya, M., Vartabedian, B.S., **Kellems, R.E.**: Metabolic and immunological consequences of limited adenosine deaminase expression in mice. *J Biol Chem,* **271**(25):15203-15210, 1996. [PMID: 8663040]

Blackburn, M.R, **Kellems R.E.**: Regulation and function of adenosine deaminase in mice. *Prog Nucleic Acid Res Mol Biol,* **55**:195-226, 1996. [PMID: 8787611]

Blackburn, M.R., Wakamiya, M., Caskey, C.T., **Kellems, R.E.**: Tissue-specific rescue suggests that placental adenosine deaminase is important for fetal development in mice. *J Biol Chem,* **270**(41):23891-23894, 1995. [PMID: 7592575]

Wakamiya, M., Blackburn, M.R., Jurecic, R., McArthur, M.J., Geske, R.S., Cartwright, J. Jr., Mitani, K., Vaishnav, S., Belmont, J.W., **Kellems, R.E.**, Finegold, M.J., Montgomery, C.A., Bradley, A., Caskey, C.T.: Disruption of the adenosine deaminase gene causes hepatocellular impairment and perinatal lethality in mice. *Proc Natl Acad Sci USA*, **92**(9):3673-3677, 1995. [PMID: 7731963]

Ingolia, D.E., Al–Ubaidi, M.R., Yeung, C.Y., Bigo, H.A., Wright, D.A., **Kellems, R.E.**: Molecular cloning of the murine adenosine deaminase gene from a genetically enriched source: Identification and characterization of the promoter region. *Mol Cell Biol,* **6**(12)**:**4458–4466, 1986. [PMID: 2432402]

Belmont, J.W., Henkel–Tigges, J., Chang, S.M., Wager–Smith, K., **Kellems, R.E.**, Dick, J.E, Magli, M.C., Phillips, R.A., Bernstein, A., Caskey, C.T.: Expression of human adenosine deaminase in murine haematopoietic progenitor cells following retroviral transfer. *Nature,* **322**(6077)**:**385–387, 1986. [PMID: 3016551]

Kaufman, R.J., Murtha, P., Ingolia, D.E., Yeung, C.Y., **Kellems, R.E.**: Selection and amplification of heterologous genes encoding adenosine deaminase in mammalian cells. *Proc Natl Acad Sci USA,* **83**(10)**:**3136–3140, 1986. [PMID: 3486414]

Ingolia, D.E., Yeung, C.Y., Orengo, I.F., Harrison, M.L., Frayne, E.G., Rudolph, F.B., **Kellems, R.E.**: Purification and characterization of adenosine deaminase from a genetically enriched mouse cell line. *J Biol Chem,* **260**(24)**:**13261–13267, 1985. [PMID: 3902813]

Yeung, C.Y., Frayne, E.G., Al–Ubaidi, M.R., Hook, A.G., Ingolia, D.E., Wright, D.W., **Kellems, R.E.**: Amplification and molecular cloning of murine adenosine deaminase gene sequences. *J Biol Chem,* **258**(24)**:** 15179–15185, 1983. [PMID: 6197412]

Yeung, C.Y., Ingolia, D.E., Bobonis, C., Dunbar, B.S., Riser, M.E., Siciliano, M.J., **Kellems, R.E.**: Selective overproduction of adenosine deaminase in cultured mouse cells. *J Biol Chem,* **258**(13)**:**8338–8345, 1983. [PMID: 6602803]

D. Research Support

Type: 1 R01 HL113574 Xia (PI) 02/01/13-01/31/17
Agency: National Institutes of Health/NHLBI
"Metabolites, sickle cell disease and novel therapeutics"
The goal is to determine the functional and structural basis for identified metabolites in sickling and disease progression.
Role: Co-I

Biographical Sketch

Provide the following information for each individual included in the Research & Related Senior/Key Person Profile (Expanded) Form.

NAME	POSITION TITLE
NAME **ANDREW W. SUBUDHI**	POSITION TITLE **ASSOCIATE PROFESSOR**

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
The Colorado College	B.A.	1992	Mathematics
Colorado State University	M.S.	1996	Exercise Science
University of Utah	Ph.D.	2000	Exercise Physiology
University of Colorado Health Science Center	Post Doc	2003-05	Altitude Physiology

RESEARCH AND PROFESSIONAL EXPERIENCE:

1997 - 2005 Research Scientist, The Orthopedic Specialty Hospital (TOSH), Intermountain Health Care, Salt Lake City, UT.
2000 - 2008 Adjunct Assistant Professor, University of Utah, Division of Foods & Nutrition, Salt Lake City, UT.
2001 - 2005 Adjunct Assistant Professor, University of Utah, Dept. of Exercise & Sport Science, Salt Lake City, UT.
2005 - 2011 Assistant Professor, University of Colorado at Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2005 - 2011 Assistant Professor, University of Colorado at Denver, Dept. of Surgery, Denver, CO.
2011-Present Associate Professor, University of Colorado at Colorado Springs, Dept. of Biology, Colorado Springs, CO.
2011-Present Associate Professor, University of Colorado Denver/Anschutz Medical Campus, Dept. of Emergency Medicine, Denver, CO.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INDIVIDUAL.

PROFESSIONAL MEMBERSHIPS

1995 – Present Member of the American College of Sports Medicine
1996 – Present Certified Strength and Conditioning Specialist (C.S.C.S.)
2000 – Present Member of the American Physiological Society

HONORS AND AWARDS

Innovations in Teaching with Technology. University of Colorado Colorado Springs, 2012
Fellow of the American College of Sports Medicine (FACSM), 2009
LAS Outstanding Teaching Award, University of Colorado at Colorado Springs, 2009
Alpha Epsilon Delta Honors Society, University of Colorado at Colorado Springs, 2006
Beta Beta Beta Honors Society, University of Colorado at Colorado Springs, 2006
Phi Kappa Phi Honors Society, University of Utah, 1999
Phi Kappa Phi Honors Society, Colorado State University, 1996
Colorado Graduate Fellowship, Colorado State University, 1995

PUBLICATIONS (from 50 total publications)

1. Hagobian T., Jacobs, K.A., Subudhi, A.W., Fattor, J.A., Rock, P.B., Muza, S.R., Fulco, C.S., Braun, B., Grediagin, A., Mazzeo, R.S., Cymerman, A., & Friedlander, A.L. (2006). Cytokine response at high altitude: effects of exercise and antioxidants at 4,300 m. Medicine and Science in Sports and Exercise, 38(2), 276-285.
2. Subudhi, A.W., Jacobs, K.A., Hagobian T.A., Fattor, J.A., Muza, S.R., Fulco, C.S., Cymerman, A., & Friedlander, A.L. (2006). Changes in ventilatory threshold at high altitude: effect of antioxidants. Medicine and Science in Sports and Exercise, 38(8), 1425-1431.
3. Amann, M., Romer, L.M., Subudhi, A.W., Pegelow, D.F., & Dempsey, J.A. (2007). Severity of arterial hypoxemia affects the relative contributions of peripheral vs. central fatigue to exercise performance. Journal of Physiology, 581(1), 389-403.
4. Subudhi, A.W. & Roach, R.C. (2008). Endurance performance at altitude. Current Sports Medicine Reports 7(1), 6-7.
5. Imray C, Wright A, Subudhi A, & Roach R. (2010). Acute mountain sickness: pathophysiology, prevention, and treatment. Progress in Cardiovascular Diseases, 52(6), 467-484.
6. Olin, J.T., Dimmen, A.C., Subudhi, A.W., & Roach, R.C. (2011). Cerebral blood flow and oxygenation at maximal exercise: The effect of clamping carbon dioxide. Respiratory Physiology & Neurobiology, 175, 176-180.
7. Asgari, S., Subudhi, A.W., Xu, P., Roach, R.C., Liebeskind, D.S., Bergsneider, B., & Hu, X. (2011). An extended model of intracranial latency facilitates non-invasive detection of cerebrovascular changes. Journal of Neuroscience Methods, 197(1), 171-179.
8. Subudhi, A.W., Dimmen, A.C., Julian, C.G., Wilson, M.J., Panerai, R.B., & Roach, R.C. (2011). Effects of acetazolamide and dexamethasone on cerebral hemodynamics in hypoxia. Journal of Applied Physiology, 110(5): 1219-1225.
9. Julian, C.G., Subudhi, A.W., Wilson, M.J., Dimmen, A.C., Pecha T., & Roach, R.C. (2011). Acute mountain sickness, inflammation and permeability: New insights from a blood biomarker study. Journal of Applied Physiology, 111(2): 392-399.

10. Subudhi, A.W., Olin J.T., Dimmen, A.C., Polaner, D.M., Kayser, B., & Roach, R.C. (2011). Does cerebral oxygen delivery limit incremental exercise performance? Journal of Applied Physiology. 111(6): 1727-1734.
11. Sato, K., Sadamoto, T., Hirasawa, A., Oue, A., Subudhi, A.W., Miyazawa, T., & Ogoh, S. (2012). Differential blood flow responses to CO2 in human internal and external carotid and vertebral arteries. Journal of Physiology, 590(Pt14): 3277-3290.
12. Olin, J.T., Dimmen, A.C., Subudhi, A.W., & Roach, R.C. (2012). A simple method to clamp end-tidal carbon dioxide during rest and exercise. European Journal of Applied Physiology, 112(9): 3439-3444.
13. Miyazawa, T., Horiuchi, M., Ichikawa, D., Subudhi, A.W., Sugawara, J., & Ogoh, S. (2012). Face cooling with water mist increases cerebral blood flow during exercise: effect of changes in facial skin blood flow. Frontiers in Physiology, 3:308.
14. Asgari, S., Gonzalez, N., Subudhi, A.W., Hamilton, R., Vespa, P., Bergsneider, M., Roach, R.C., & Hu, X. (2012). Continuous detection of cerebral vasodilatation and vasoconstriction using intracranial pulse morphological template matching.PLoS One, 7(11):e50795.
15. Schommer, K., Menold, E., Subudhi, A.W., Bartsch, P. (2012). Health risks for athletes at moderate altitude and normobaric hypoxia. British Journal of Sports Medicine, 46(11): 828-32.
16. Lundby, C., Millet, G.P., Calbet, J.A., Bartsch, P., & Subudhi, A.W. (2012). Does 'altitude training' increase exercise performance in elite athletes? British Journal of Sports Medicine, 46(11): 792-795.
17. Bergeron, M.F., Bahr, R., Bartsch, P., Bourdon, L., Calbet, J.A., Carlsen, K.H., Castagna, O., Gonzalez-Alonso, J., Lundby, C., Maughan, R.J., Millet, G., Mountjoy, M., Racinais, S., Rasmussen, P., Singh, D.G., Subudhi, A.W., Young, A.J., Soligard, T., & Engebretsen, L. (2012). International Olympic Committee consensus statement on thermoregulatory and altitude challenges for high-level athletes. British Journal of Sports Medicine, 46(11): 770-779.
18. Ogoh, S., Sato, K., Nakahara, H., Okazaki, K., Subudhi A., & Miyamoto, T. (2013). Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. Experimental Physiology. 98.3: 692-698.
19. Amann, M., Goodall, S., Twomey, R., Subudhi, A.W., Lovering, A.T., & Roach, R.C. (2013). AltitudeOmics: On the consequences of high altitude acclimatization for the development of fatigue during locomotor exercise in humans. Journal of Applied Physiology. 115(5): 634-642.
20. Goodall S., Twomey R., Amann M., Ross E.Z., Lovering A.T., Romer L.M., Subudhi A.W., Roach R.C. (2014). AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatisation to high altitude. Atca Physiologica (Oxford), 210(4):875-88.
21. Julian, C.G., Subudhi, A.W., Hill, R.C., Wilson, M.J., Dimmen, A.C., Hansen, K.C., & Roach, R.C. (2014). Exploratory proteomic analysis of hypobaric hypoxia and acute mountain sickness and in humans. Journal of Applied Physiology, 116(7):937-44.
22. Fan, J.L., Subudhi, A.W., Evero, O., Bourdillon, N., Kayser, B., Lovering, A.T., & Roach, R.C. (2014). AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high altitude acclimatisation and re-exposure. Journal of Applied Physiology, 116(7):911-8.
23. Subudhi, A.W., Fan, J.L., Evero, O., Bourdillon, N., Kayser, B., Julian, C.G., Lovering, A.T., Panerai, R.B., & Roach, R.C. (2014). AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. Journal of Applied Physiology, 116(7):724-9.
24. Subudhi AW, Bourdillon N, Bucher J, Davis C, Elliott JE, Eutermoster M, Evero O, Fan JL, Jameson-Van Houten S, Julian CG, Kark J, Kark S, Kayser B, Kern JP, Kim SE, Lathan C,

Laurie SS, Lovering AT, Paterson R, Polaner D, Ryan BJ, Spira J, Tsao JW, Wachsmuth NB, Roach RC (2014). AltitudeOmics: the integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent. PLoS One; 9(3):e92191

25. Subudhi, A.W., Fan, J.L., Evero, O., Bourdillon, N., Kayser, B., Julian, C.G., Lovering, A.T., & Roach, R.C. (in press). AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. Experimental Physiology, 99(5):772-81.
26. Ogoh S., Nakahara, H., Ueda, S., Okazaki, K., Shibasaki, M., Subudhi, A., Miyamoto, T. (2014). Effects of acute hypoxia on cerebrovascular responses to carbon dioxide. Experimental Physiology, 99(6):949-8
27. Ainslie, P.N. & Subudhi, A.W. (2014). Cerebral blood flow at high altitude. High Altitude Medicine and Biology, 15(2):133-40.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
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NAME	POSITION TITLE
Yujin Zhang	Research Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Medical School, Wuhan University of Science and Technology, Wuhan, P. R. China	BS	07/84	Medicine
Tongji Medical University, Wuhan, P.R. China	MS	07/91	Internal Medicine
Tongji Medical University, Wuhan, P.R. China	PhD	07/96	Hematology

A. Personal statement

As an outstanding research scientist at department of biochemistry and molecular biology, I have intensive experience in molecular and cellular biology. In particular, I am very familiar with HPLC to measure adenosine levels in the blood and tissues in both mouse and human.

B. Positions and Honors

1984-1988	Resident, 701 Hospital in Shangrao city, China.
1991-1993	Resident of the second hospital of Wuhan city, China.
1996-1999	Physician-in-charge, Dept. of hematology, Union hospital, Tongji Medical University, Wuhan 430030, China.
1996-1999	Research Fellow, The Institute of Hematology, Tongji Medical University, Wuhan, P.R. China.
1999-2000	Postdoctoral fellow at Dept. of Surgery, The University of Texas Medical Branch, Galveston, TX
2000-2002	Postdoctoral Associate at Dept of pathology, Baylor College of Medicine, Houston, TX
2002-2006	Postdoctoral Associate at Dept of ophthalmology, The University of Texas-Houston Medical School, Houston, TX.
2006-2010	Research Scientist at Dept of Biochemistry & Molecular Biology, The University of Texas-Houston Medical School, Houston, TX.
2010-present	Research Assistant Professor at Dept of Biochemistry & Molecular Biology, The University of Texas-Houston Medical School, Houston, TX.

C. Selected Peer-reviewed Publications

1. **Zhang, YJ.,** S.F. Schlossman, R.A. Edwards, Ching-Nan Ou, J. Gu, and Wu, XM. Impaired apoptosis, extended duration of immune responses, and a lupus-like autoimmune disease in IEX-1-transgenic mice. **PNAS 99**, 878-883 (2002). PMID:**11782530**

2. Huang, Y-H, Wu,JM., **Zhang, YJ.,**and Wu, MX., Synergistic and opposing regulation of the stress-responsive gene IEX-1 by P53, c-Myc, and multiple NF-kB/rel complexes. **Oncogene 21**, 6819-6828. (2002). PMID:14534530

3 **Zhang, YJ.,** Finegold, MJ., Kanteti, P., Chu, F. and Wu.MX, Development of T cell lymphoma in Eμ-IEX-1 mice. **Oncogene 9**, 6845-51 (2003). PMID:14534530

4. **Zhang YJ.,** Finegold MJ, Jin Y, **Wu** MX. Accelerated transition from the double-positive to single-positive thymocytes in Galphai2-deficient mice **Int Immunol. 17**, 233-43 (2005). PMID: 15684040

5. Zhou, C.C**., Zhang, YJ**., Irani, R.A., Zhang, H., Mi, T., Popek, E.J., Hicks, M.J., Ramin, S.M., Kellems, R.E. & Xia, Y**.** Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice ***Nature Medicine* 14**, 855-862 (2008). PMID:18660815

6. Dai, Y., **Zhang, YJ**., Phatarpekar, P., Mi, T., Zhang, H., Blackburn, M.R. & **Xia, Y.** Adenosine signaling, priapism and novel therapies ***The Journal of Sexual Medicine* 6 Suppl 3**, 292-301 (2009). PMID:19267852

7. Irani, R.A., **Zhang, YJ**., Blackwell, S.C., Zhou, C.C., Ramin, S.M., Kellems, R.E. & Xia, Y. The detrimental role of angiotensin receptor agonistic autoantibodies in intrauterine growth restriction seen in preeclampsia ***The Journal of Experimental Medicine* 206**, 2809-2822 (2009). PMID:19887397

8. Wen, J., Jiang, X., Dai, Y., **Zhang, YJ**., Tang, Y., Sun, H., Mi, T., Kellems, R.E., Blackburn, M.R. & Xia, Y. Adenosine deaminase enzyme therapy prevents and reverses the heightened cavernosal relaxation in priapism ***The Journal of Sexual Medicine* 7**, 3011-22 (2010). PMID:19845544

9. Wen, J., Jiang, X., Dai, Y., **Zhang, YJ**., Tang, Y., Sun, H., Mi, T., Phatarpekar, P.V., Kellems, R.E., Blackburn, M.R. & Xia, Y. Increased adenosine contributes to penile fibrosis, a dangerous feature of priapism, via A2B adenosine receptor signaling ***FASEB J* 24**, 740-9 (2010). PMID:19858092

10. Zhou, C.C., Irani, R.A., **Zhang, YJ**., Blackwell, S.C., Mi, T., Wen, J., Shelat, H., Geng, Y.J., Ramin, S.M., Kellems, R.E. & Xia, Y. Angiotensin receptor agonistic autoantibody-mediated tumor necrosis factor-{alpha} induction contributes to increased soluble endoglin production in preeclampsia ***Circulation* 26**, 121(3):436-44 (2010). PMID:20065159

11. Irani, RA, **Zhang, YJ**, Zhou, CC, Blackwell, SC, Hicks, JM,. Ramin, SM, Kellems, RE and Xia, Y**.** Autoantibody-mediated angiotensin receptor activation contributes to preeclampsia through TNF-alpha signaling ***Hypertension***;55, 1246-53 (2010). PMID:20351341

12. Dai, YB, Zhang, WR, **Zhang, YJ**., Wen, JM, Kellems, RE and Xia, Y**.** A2B adenosine receptor-mediated induction of IL-6 promotes CKD ***Journal of American Society of Nephrology*** 22, 890-901 (2011) PMID: 21511827

13. **Zhang, YJ**, Dai, YB, Wen, JM, Grenz, A., Sun, H, Tao, LJ, Lu, GX, Danny, AC, Milburn, Michael V, Louvenia, Carter-Dawson, Lewis, DE, Zhang, WZ , Kellems, RE, Eltzschig, H. Blackburn, MR, Juneja, HS. and Xia, Y**.** Detrimental effects of adenosine signaling in sickle cell disease ***Nature Medicine* 17**, 79-86 (2011). PMID:21170046

14. Wen, JM, Dai, YB, **Zhang, YJ**., Zhang, WR, Kellems, RE, Blackburn, MR, Eltzschig, HK and Xia, Y. Impaired erectile function in CD73-deficient mice with reduced intrinsic penile adneosine production ***Journal of Sexual Medicine*** [Epub ahead of print] (2011) PMID: 21595838

15. Wen, JM, Grenz, A, Dai, YB, **Zhang, YJ**., Kellems, RE, Blackburn, MR, Eltzschig, HK and Xia, Y**.** A_{2B} adenosine receptor contributes to penile erection via PI3K/AKT signaling cascade-mediated eNOS activation ***FASEB J*** [Epub ahead of print] (2011) PMID: 21566208

16. **Zhang, YJ**, Xia Y. Adenosine signaling in normal and sickle erythrocytes and beyond. Microbes and Infection. 2012 .

17. Wang, W, Irani, RA, **Zhang, YJ**, Ramin, SM, Blackwell, SC, Tao, LJ, kellems, RE, Xia Y. Autoantibody-mediated complement C3a receptor activation contributes to the pathogenesis of preeclampsia. **Hypertension** 2012 ; 60(3) :712-21.

18. Ning, C, Qi, L, Wen, JM, **Zhang**, YJ, Zhang, WR, Wang, W, Blackburn, MR, Kellems, RE, Xia Y. Excessive Penile Norepinephrine Level Underlies Impaired Erectile Function in Adenosine A_1 Receptor Deficient Mice. **J. Sex Med**. 2012; 9(10) 2552-2561.

19. Zhang W, **Zhang Y**, Wang W, Dai Y, Ning C, Luo R, Sun K, Glover L, Grenz A, Sun H, Tao L, Zhang W, Colgan SP, Blackburn MR, Eltzschig HK, Kellems RE, Xia Y. Elevated ecto-5'-nucleotidase-mediated increased renal adenosine signaling via A2B adenosine receptor contributes to chronic hypertension. **Circulation Research** 2013; 112(11):1466-78.

20. Wei Wang, Nicholas Parchim, Takayuki Irima, Renna Luo, Cheng Zhao, Chen Liu, Roxanna Irani, Weiru Zhang, Chen Ning, **Yujin Zhang**, Sean Blackwell, Lieping Chen, Lijian Tao, John Hicks, Rodney Kellems, and Yang Xia. Excess LIGHT contributes to placental impairment, toxic factor secretion, hypertension and proteinuria in preeclampsia. **Hypertension** (in press).

21. **Yujin Zhang**, Vladimir Berka, Anren Song, Kaiqi Sun, Wei Wang, Weiru Zhang, Chen Ning, Chonghua Li, Qibo Zhang, Mikhail Bogdanov, Danny C. Alexander, Michael V. Milburn, Mostafa H. Ahmed, Han Lin, Modupe Idowu, Jun Zhang, Gregory J. Kato, Osheiza Y. Abdulmalik, Wenzheng Zhang, William Dowhan, Rodney E. Kellems, Pumin Zhang, Jianping Jin, Martin Safo, Ah-Lim Tsai, Harinder S. Juneja and Yang Xia. Elevated sphingosine-1-phosphate promotes sickling and sickle cell disease progression. J.Clin.Invest. 2014 124(6): 2750-2761.

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
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NAME Michael R. Blackburn	POSITION TITLE Dean and Professor
eRA COMMONS USER NAME (credential, e.g., agency login) MBLACKBURN	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
King College, Bristol, TN	B.S.	1984-88	Biology/Chemistry
Thomas Jefferson University	Ph.D.	1988-93	Developmental Biology
Baylor College of Medicine	Post Doc	1993-97	Molecular Genetics

A. Personal Statement

My area of research expertise is in the analysis of adenosine signaling in the pathogenesis of acute lung injury and fibrotic lung diseases. The majority of my work has investigated the role of adenosine signaling in lung diseases such as asthma, COPD and idiopathic pulmonary fibrosis (IPF); however, I have published experience in examining fibrotic models in the skin, kidney and liver as well. Our overall hypothesis is that the adenosine generated during injury contributes to the progression disease (tissue remodeling, destruction and fibrosis) by promoting an overactive wound healing response. I have generated or collected genetically modified mice or selective pharmaceutical reagents that allow us to address important roles of adenosine signaling pathways in *in vivo* models. In addition, we have active programs investigating systems biology approaches to understanding lung disease and the role of mRNA processing in lung disease. We utilize molecular, cellular and physiological approaches to pursue specific mechanistic questions and conduct proof of concept experiments in tissues and cells derived from patients with acute lung injury and various fibrotic lung diseases including IPF. Along these lines, I run a tissue bank for explanted human lung tissue supported by local hospitals in the Texas Medical Center. In addition, I have extensive experience in reviewing manuscripts and grants in these disease areas, and have active collaborations with industry entities that are developing novel compounds for the treatment of lung disease.

B. Positions and Honors

Professional Experience

1997-2003	Assistant Professor, Department of Biochemistry and Molecular Biology University of Texas Medical at Houston, Houston TX
2003-2006	Associate Professor, Department of Biochemistry and Molecular Biology University of Texas Medical School at Houston, Houston TX
2006-present	Professor, Department of Biochemistry and Molecular Biology University of Texas Medical School at Houston, Houston TX
2005-2010	Director, Graduate Program in Biochemistry and Molecular Biology University of Texas Medical School at Houston, Houston TX
2011-present	Vice Chairman, Department of Biochemistry and Molecular Biology University of Texas Medical School at Houston, Houston TX
2012-present	Dean, The University of Texas Graduate School of Biomedical Science at Houston

Honors and Awards

1994 - 1997	NIH, National Research Service Award
2000	Sandler Program for Asthma Research Young Investigator Award
2004	American Lung Association Career Investigator Award

2005-2010	UT Medical School, Dean's Teaching Excellence Award
2011	Paul Darlington Outstanding Mentor Award2012Member,
2012	The University of Texas Academy of Health Science Education
2012	John P. McGovern Distinguished Professor of Biomedical Sciences, The University of Texas Graduate School of Biomedical Sciences at Houston
2013	UT Health Presidents Scholar Award for Research
2014	William S. Kilroy Sr., Chair in Pulmonary Disease

Service on NIH study Sections

(1999) PO1 "Asthma and Allergic Disease Research Centers"; (2000) Idea Grant Review Section; (2001) PO1 "Regulation of Lung Inflammation"; (2002) PO1 "Lung Inflammation Research Centers"; (2006) SCCOR, "Lung Host Diseases"; (2006) Lung Cellular and Molecular Immunology Study Section, Ad Hoc; (2007) Lung Injury, Repair and Remodeling Study Section, Ad Hoc; (2008-2012) Lung Injury, Repair and Remodeling Study Section, Regular Member

C. Selected Peer-reviewed Publications (Selected from 135)

1. **Blackburn, M. R.**, Volmer, J. B., Thrasher. J. C., Crosby, J. R., Lee, J. J. and Kellems, R. E. (2000) Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation and airway obstruction. *J. Exp. Med*. 192, 159-170. PMCID: PMC2193256
2. **Blackburn, M. R.**, Chun, G. L., Young, H. W. J., Zhu, Z., Chunn, J. L., Kang, M. J., Banerjee, S. K., and Elias, J. A. (2003) Adenosine mediates IL-13-induced inflammation and remodeling in the lung and interacts in an IL-13-adenoisne amplification pathway. *J. Clin. Invest.* **112,** 332-344. PMCID: PMC166289
3. Sun, C.-X., Young, H. W., Molina, J. G., Volmer, J. B., Schnermann, J. and **Blackburn, M. R.** (2005) A protective role for the A1 adenosine receptor in adenosine-dependent pulmonary injury. *J. Clin. Invest.* **115**, 35-43. PMCID: PMC539198
4. Sun, C.-X., Young, H. W., Molina, J. G., Volmer, J. B., Schnermann, J. and **Blackburn, M. R.** (2005) A protective role for the A1 adenosine receptor in adenosine-dependent pulmonary injury. *J. Clin. Invest.* **115**, 35-43. PMID: 15630442
5. Chunn, J. L., Molina, J. G., Mi, T., Xia, Y., Kellems, R. E. and **Blackburn, M. R.** (2005) Adenosine dependent-pulmonary fibrosis in adenosine deaminase-deficient mice. *J. Immunol.* **175,** 1937-1946. PMID: 16034138
6. Sun, C. X., Zhong, H., Mohsenin, A., Morschl, E., Chunn, J. L., Molina, J. G., Belardinelli, L., Zeng, D. and **Blackburn, M. R.** (2006) Role of A_{2B} adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury in adenosine deaminase deficient mice. *J. Clin. Invest.* **116,** 2173-2182. PMCID: PMC1501110
7. Young, H. W. J., Sun, C.-X., Evans, C. M., Jacobson, M. A., Dickey, B. and **Blackburn, M. R.** (2006) A3 adenosine receptor signaling contributes to airway mucin secretion following allergen challenge. *Am. J. Respir. Cell Mol. Biol.* **35,** 549-558. PMCID: PMC2643274 (Featured on Journal Cover).
8. Morschl, E., Molina, J. G., Volmer, J., Mohsenin, A., Pero, R. S., Hong, J. S., Kheradmand, F., Lee, J. J. and **Blackburn, M. R.** (2008) A3 adenosine receptor signaling influences pulmonary inflammation and fibrosis. *Am. J. Respir. Cell Mol. Biol.* **39,** 697-705. PMCID: PMC2586045 (Featured on Journal Cover)
9. Zhou, Y., Mohsenin, A., Morschl, E., Young, H. W. J., Molina, J. G., Ma, W., Sun, C. X., Martinez-Valdez, H. and **Blackburn, M. R.** (2009) Enhanced airway inflammation and remodeling in adenosine deaminase deficient mice lacking the A2B adenosine receptor. *J. Immunol.* **182,** 8037-8046. PMCID: PMC3631106
10. Zhou, Y., Murthy, J., Zeng, D., Belardinelli, L. and **Blackburn, M. R.** (2010) Alterations in adenosine metabolism and signaling in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *PloS ONE* **5,** 3 9224**.** PMCID: PMC2821921
11. Zhou, Y., Schneider, D. J., Morschl, E., Song, L., Pedroza, M., Karmouty-Quintana, H., Le, T., Sun, C.X. and **Blackburn, M. R.** (2011) Distinct roles for the A2B adenosine receptor in acute and chronic stages of bleomycin-induced lung injury. *J. Immunol.* **186,** 1097-1106. PMCID: PMC2607290

12. Karmouty-Quintana, H., Zhong, H., Acero, L., Weng, T., Melicoff, E., West, D. W., Hemnes, A., Grenz, A., Eltzschig, H. K., Blackwell, T. S., Xia, Y., Johnston, R. A., Zeng, D., Belardinelli, L. and **Blackburn, M. R.** (2012) The A2B adenosine receptor modulates pulmonary hypertension associated with interstitial lung disease. *FASEB J*. **26,** 2546-2557. PMCID: PMC3650483
13. Weng, T., Karmouty-Quintana, H., Garcia-Morales, L. J., Molina, J. G., Pedroza, M., Bunge, R. R., Bruckner, B. A., Loebe, M., Seethamraju, H. and **Blackburn, M. R.** (2013) Hypoxia-induced deoxycytidine kinase expression contributes to apoptosis in chronic lung disease. *FASEB J.* **27,** 2013-2026. PMCID: PMC4046114
14. Karmouty-Quintana, H., Weng, T., Garcia-Morales, L. J., Chen, N. Y., Pedroza, M., Zhong, H., Molina, J. G., Bunge, R., Bruckner, B. A., Xia, Y., Johnston, R. A., Loebe, M., Zeng, D., Seethamaraju, H., Belardinelli, L. and **Blackburn, M. R.** (2013) ADORA2B and hyaluronan modulate pulmonary hypertension associated with chronic obstructive pulmonary disease. *Am. J. Respir. Cell. Mol. Biol.* **49,** 1038-1047.
15. Le, T. T. T., Karmouty-Quintana, H., Melicoff, E., Le, T. T. T., Weng, T., Chen, N. Y., Pedroza, M., George, A. T., Garcia-Morales, L. J., Bunge, R. R., Bruckner, B. A., Loebe, M., Seethamraju, H., Agarwal, S. K. and **Blackburn, M. R.** (2014) Blockade of interleukin-6 trans signaling attenuates pulmonary fibrosis. *J. Immunol.* **193,** 3755-3768. PMCID: PMC4169999 (Featured on Journal Cover)

D. Research Support

ONGOING

2 RO1 HL70952 (Blackburn) 08/01/11-06/30/15
Adenosine Signaling and Lung Fibrosis NIH/NHLBI
The goals of this study are to 1) examine A2BR-dependent IL-6 production as a mechanism for promoting pulmonary fibrosis; 2) examine mechanisms by which IL-6 influences pulmonary fibrosis; and 3) examine A2BR-mediated IL-6 production, IL-6R shedding and STAT-3 activation in IPF patients.
Role: PI

1 PO1 HL114457 (PD, Blackburn) 06/01/13-05/31/18
Hypoxic Adenosine Responses NIH/NHLBI
The goal of this program project is to define the pathways by which adenosine generation and signaling is beneficial in acute lung and kidney injury and detrimental during chronic lung disease (fibrosis) and sickle cell disease.
Role: Program Director

Project 1. *Hypoxic Adenosine Responses in the Regulation of Lung Injury.* (PI, Blackburn)
The goal of this project is to examine the mechanisms underlying the protective effects of adenosine during acute lung injury, the detrimental effects of adenosine in chronic lung disease and the continuum between the two. The focus will be on the promotion of barrier function by adenosine during acute lung injury and the role of adenosine-dependent alternatively activated macrophages during fibrotic disease stages.

Leader of Core A. *Administrative Core*. (PI, Blackburn)
The goal of this core will be to provide the administrative infrastructure needed to promote interactions between component project of this PPG.

PO 4500020593/GS-6201 (Blackburn) 02/20/04-07/30/15
Gilead Sciences/CV Therapeutics Inc.
Analysis of the A_{2B} adenosine receptor in adenosine mediated lung disease
The goal of this project is to examine the contribution of A_{2B} adenosine receptor signaling in adenosine-mediated fibrosis in ADA-deficient mice and the bleomycin model using selective A_{2B} receptor antagonists provided by Gilead. These studies provided preclinical data that led to the FDA filing of CVT-6883 for the treatment of pulmonary fibrosis.
Role: PI

UL1TR000371 (McPherson) 06/27/12-05/31/17
NIH/National Center for Advancing Translational Sciences
Center for Clinical and Translational Sciences (CCTS)
The goal of the CCTS is to move scientific and medical discoveries as fast as possible from the laboratory to the clinic and community, where they can improve the health of the American people. The CCTS trains researchers, provides research services, and works with its communities to learn their health concerns and spread health care information.
Role: Co-director of the TL1 component of the CTSA award

1 R01HL113574 (Xia) 02/01/13-01/31/17
Metabolites, Sickle Cell Disease, and Novel Therapeutics NIH/NHLBI
The goal of the proposed research is the development of novel approaches to ameliorate erythrocyte sickling in individuals with sickle cell disease (SCD).
Role: Co-I

1 R01 AI077679 (Shyu) 07/25/11-06/30/15
Translational Regulation in Bronchial Epithelial Cells NIH/NIAID
The aims of this proposal are to determine whether a reduction in miR-26 and miR-16 abundance contributes to the persistent, elevated level of IL-6 observed in asthmatic primary HBE cells; to define the role of a group of miRNAs that are significantly down-regulated in asthmatic primary HBE cells in controlling the activity of translation machinery in bronchial epithelial cells; and to determine whether a reduction in P-bodies is a hallmark of activated bronchial epithelial cells, and how alteration of P-body assembly and disassembly influences the inflammatory response in bronchial epithelial cells.
Role: Co-PI

PR110864 (Agarwal) 09/30/12-09/29/15
DOD/Baylor College of Medicine
Cadherin-11 Regulation of Fibrosis through Modulation of Epithelial-to-mesenchymal Transition: Implications for pulmonary fibrosis in scleroderma
The aims of this proposal are to determine the contribution of cadherin-11 to process of epithelial-to-mesenchymal transition in airway epithelial cells, to investigate the expression of cadherin-11 on alveolar macrophages and the cadherin-11 dependent regulation of TGF-beta production by alveolar macrophages and to determine if cadherin-11 is a key mediator of fibrosis in the intraperitoneal bleomycin model of pulmonary fibrosis and if cadherin-11 modulates epithelial-to-mesenchymal transition in vivo during the development of pulmonary fibrosis.
Role: Subaward Co-I

<u>COMPLETED</u>

2 R01 DK056804 (Fallon) 08/01/10-07/31/14
NIH/NIDDK
Mediators of Pulmonary Vasodilatation in Liver Disease
The long-term goal is to use an understanding of vascular dysfunction in HPS to develop medical therapies and as a paradigm for understanding the pathogenesis of other vascular complications of liver.
Role: Co-I

1 R01 DK083559 (Xia) 05/01/09-03/31/14
Adenosine Signaling, Priapism and Sickle Cell Disease
The major goal of this study is to reveal an important role for adenosine signaling in several aspects of the penile erection process and highlight various therapeutic opportunities to treat priapism and other erectile disorders.
Role: Co-I

PREVIOUS/CURRENT/PENDING SUPPORT

Xia, Y.

PREVIOUS (ending within the last 5 years)

"Adenosine Signaling, Priapism and Sickle Cell Disease"
1 R01 DK083559 (Xia) 5/1/09-3/31/13 3.0 calendar months
National Institutes of Health
Contact: Diana T Ly, 6707 Democracy Blvd., BG 2DEM RM 723, Bethesda, MD 20817
The goal was to determine the molecular mechanisms for adenosine-induced priapism in sickle cell disease.
Specific Aims: 1) What are the intracellular targets and signaling pathways involved in excess adenosine-induced priapism? 2) What are the molecular mechanisms generating excess adenosine in priapism? 3) What are the sources of adenosine in the penis and what regulates its production during initiation and maintenance of normal penile erection?

"Sickle Cell Anemia, Vascular Endothelial Dysfunction and Novel Therapeutics"
12IRG9150001 (Xia) 01/01/12-12/31/13 1.2 calendar months
American Heart Association
Contact: Alma Cooks, 7272 Greenville Avenue, National Center, Dallas, Texas 75231-4596
The goal of the proposed research is the development of novel approaches to ameliorate vascular endothelial dysfunction and prevent multiple life-threatening complications associated with sickle cell anemia including pulmonary hypertension (PH) and stroke.
Specific Aims: 1) Determine if S1P and Ado are novel pathogenic biomarkers correlated with disease severity (AVE or PH) in individuals with SCA; 2) Determine whether Ado and S1P are safe and effective therapeutic targets to reduce morbidity and mortality in SCA mice.

"Autoantibodies in Preeclampsia: Pre-symptomatic markers and therapeutic targets"
1 RC4 HD067977 (Kellems & Xia) 09/27/10-09/30/13 3.0 calendar months
National Institutes of Health)
Contact: Can Varol, 31 Center Drive, Bldg. 31, Room 2A32, Bethesda, MD 20892-2425
The goal of this study is to develop biological and immunological test to identify AT1-AA as a pre-symptomatic risk factor for PE and to develop therapeutic strategies to prevent or treat PE based on blocking the pathophysiological consequences of autoantibody-induced angiotensin receptor activation.
Specific Aims: 1) Develop pre-symptomatic testing based on early detection of angiotensin receptor activating autoantibodies (AT1-AAs) associated with preeclampsia; 2) Develop therapeutic strategies to prevent or treat preeclampsia based on blocking autoantibody-induced angiotensin receptor activation.

"Autoantibody-Induced Inflammatory Response Underlies the Pathogenesis of Preeclampsia"
10GRNT3760081 (Xia) 07/01/10-06/30/12 1.2 calendar months
American Heart Association
Contact: Alma Cooks, 7272 Greenville Avenue, National Center, Dallas, Texas 75231-4596
The goal was to determine whether increased inflammatory response underlies autoantibody-induced preeclampsia.
Specific Aims: 1) Assess the exact role of AT1-AA-induced inflammatory response in PE; 2) Determine the molecular basis of AT1-AA-induced inflammatory cascade in PE.

"Preeclampsia and Autoimmunity"
2 R01 HD034130 (Kellems) 02/15/08-01/31/13 1.8 calendar months
National Institutes of Health
Contact: Grace Poe, 6100 Executive Blvd., BG 6100 RM 8A17H, Rockville, MD 20852

The goal was to determine how angiotensin receptor agonistic autoantibodies cause preeclampsia.
Specific Aims: 1) Evaluate the potential contribution of AT1-AAs to the pathophysiology of preeclampsia; 2) Examine therapeutic strategies based on blocking autoantibody-induced AT1 receptor activation; 3) Determine the mechanism of AT1-AA-induced AT1 receptor activation.
Role: Co-Investigator

CURRENT

"Metabolites, Sickle Cell Disease, and Novel Therapeutics"
1 R01 HL113574 (Xia) 02/01/13-01/31/17 2.4 calendar months
National Institutes of Health
Contact: Kevin Heath, 6701 Rockledge Dr., RKL2 BG RM 7165, Bethesda, MD 20817
The goal of the proposed research is to determine newly identified metabolites in sickle cell disease and develop new therapies for the disease.
Specific Aims: 1) Determine the molecular basis underlying A2BR-mediated induction of 2,3-DPG in erythrocytes; 2) Determine pathogenic mechanisms underlying elevated S1P-induced sickling, inflammation and progression; 3) Determine molecular mechanisms responsible for the elevation of S1P in SCD; 4) Determine if elevated Ado, S1P and 2,3-DPG are novel pathogenic biomarkers that correlate to disease severity and phenotypic variation.
Overlap: No overlap with the proposed project.

"Hypoxic Adenosine Responses"
Project 3: Novel Roles of the Erythrocyte in the Hypoxic Adenosine Response
1 P01 HL114457 (Blackburn) 06/01/13-05/31/18 3.0 calendar months
NIH
Contact: John Bucheimer, 6701 Rockledge Dr., RKL2 BG RM 7136, Bethesda, MD 20817
The goal is to assess the role of erythrocyte function in hypoxia-mediated elevation of adenosine in tissue injury.
Specific Aims: 1) Extend our discovery of detrimental effects of elevated adenosine signaling in SCD to preclinical animal studies and human translational studies; 2) Define the importance of ADORA2B signaling in normal and SCD erythrocytes in hypoxia-induced acute tissue injury; 3) Evaluate the role of ENTs on normal and SCD erythrocytes in hypoxia-induced tissue injury.
Role: Project 3 Leader
Overlap: No overlap with the proposed project.

PENDING

"Adenosine Signaling in Priapism and Erectile Dysfunction and Novel Therapies"
2 R01 DK083559 (Xia) 04/01/15-03/31/20 3.0 calendar months
National Institutes of Health
Contact: This proposal was submitted early July 2014. A grants officer has not been assigned.
The major goal of this study is to provide significant new insight regarding priapism and ED pathogenesis and in turn provide new therapeutic options for safe and effective treatment.
Specific Aims: 1) Determine molecular basis underlying priapism in ENT2-deficient mice and the importance of reduced penile ENT2 in priapism in SCD mice; 2) Define novel role of erythrocyte ADORA2B-mediated O2 release in normal erection, priapism and ED; 3) Conduct translational studies to determine if circulating Ado and 2,3 levels of these pathogenic metabolites are associated with the phenotypic variation of priapism in SCD patients.
Overlap: No overlap with the proposed project.

“Blood Oxygen Supply and Diet-induced Obesity”
Pending (Tong) 07/01/15-06/30/20 0.6 calendar months
National Institutes of Health
Contact: This proposal was submitted early October 2014. A grants officer has not been assigned.
The goal of the proposed research is to reveal whether RBC O2 supply physiologically regulates energy expenditure, validate the novel hypothesis that defective response in promoting RBC O2 supply to HFD-induced SNS activity contributes significantly to the current obesity epidemic and provide a basis for using RBC O2 supply as a novel target to predict, prevent and reverse obesity.
Specific Aims: 1) To test whether β-ARs on RBCs are required to regulate O2 supply, determine EE and mediate resistance to DIO; 2) To test whether increasing RBC O2 supply is sufficient to increase EE and promote resistance to diet-induced obesity; 3) To test whether naturally occurring variable blood O2 content contributes to differential susceptibility to diet-induced obesity.
Role: Collaborator
Overlap: No overlap with the proposed project.

PREVIOUS/CURRENT/PENDING SUPPORT

Roach, R.

PREVIOUS (ending within the last 5 years)

AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude

W81XWH-11-2-0040 (Roach, PI) 01/2011-6/2014 (publications are still ongoing)
DMRDP
Contact: Jason Ghannadian, Telemedicine and Advanced Technology Research Center (TATRC)
Bldg. 1054 Patchel Street Fort Detrick, Maryland 21702-5012. (301) 619-0235

This project aims at advancing high-altitude medical research by discovering the basic molecular mechanisms of acclimatization that protect soldiers from high altitude illness.

No overlap with the proposed project.

CURRENT

"Prediction of acute mountain sickness using a blood-based test"
W81XWH-11-2-0034 (Roach, PI) 12/2010-12/2015 4.5 calendar
DMRDP
Contact: Jason Ghannadian, Telemedicine and Advanced Technology Research Center (TATRC)
Bldg. 1054 Patchel Street Fort Detrick, Maryland 21702-5012. (301) 619-0235

This project aims at developing a rapid, cost-effective, pre-ascent screening test to predict individual risk of acute mountain sickness (AMS) for military use.

No overlap with the proposed project.

"Combinational Drug Screening to Identify Strategies That Enhance Ground Troop Readiness at Altitude"
Contract # N66001-10-C-2134 (Irwin Pi, Roach Co-PI) 02/11-05/2015 4 calendar
DARPA
Contact: Shannon Kasa, SSC P Code 71510, San Diego, CA 92152 (619) 553-3889
The advancement of high-altitude medical research by discovering novel preventive measures for acute mountain sickness.

No overlap with the proposed project.

PENDING

"Three New Ideas to Protect the Special Forces From the Stress of High Altitude"
W81XWH-USSOCOM-BAA-14-1 (Roach, PI) 2.5 calendar
USSOCOM
This project will test the efficacy of three compounds we believe will prevent acute mountain sickness (AMS) and optimize performance of special operations forces (SOF) at operationally relevant altitudes of 10,000 to 13,000 feet. To achieve this objective we will select 60 men living at sea level who meet or exceed basic physical fitness requirements for SOF. These subjects will be assigned to one of four treatment groups (placebo, quercetin, nifedipine+ methazolamide, or metformin) and transported to high altitude to test the efficacy of the treatments on symptoms of acute mountain sickness (AMS) and physical and cognitive performance over a three-day period.

Overlap: some minor apparent overlap in that the same drug is tested. However, the aims of both projects are different in since this project exclusively analyzes performance whereas the current proposal analyzes oxygen transport.

John Davis Current and Pending Support

<u>CURRENT</u>

"PRISM: Positive Routes Into Science and Mathematics"
NSF #856613 09/15/09- 09/01/15 1.0 calendar
NSF-STEP
Contact: Lee Zia lzia@nsf.gov, 703-292-8670

The PRISM project is designed to increase the number of STEM graduates through a positive retention program that creates a first-year STEM learning community and engages students by a focus on undergraduate research in the first year of study.

Overlap: No overlap with the proposed project.

"e-STEM: Enhancing STEM Education and Practice"
Dow Foundation 07/15/14- 09/01/16 1.0 calendar

Contact: Macauley Whiting, President Dow Foundation, 1018 West Main Street, Midland, Michigan 48640

The goal of e-STEM is to increase student interest in STEM fields by enhancing opportunities at Alma College and K-12 schools to engage in STEM research. Its objectives include improving K-12 STEM pedagogy by facilitating best practices and access to equipment, and enriching K-16 STEM education by responding to local needs.

Overlap: No overlap with the proposed project.

<u>PENDING</u>

"The Science Scholars Program"
NSF 06/01/15 – 06/01/18 .5 calendar
NSF S-STEM
NSF Contact: No program officer has been assigned.

The Alma College Science Scholars program will increase the number and quality of STEM graduates through a scholarship program designed to support and encourage STEM students as they achieve success in the classroom and success in the research laboratory, preparing themselves for the next step in their careers.

Overlap: No overlap with the proposed project

PREVIOUS/CURRENT/PENDING SUPPORT

Kellems, R.

PREVIOUS (ending within the last 5 years)

"Autoantibodies in Preeclampsia: Pre-symptomatic markers and therapeutic targets"
1 RC4 HD067977 (Kellems & Xia) 09/27/10-09/30/13 3 calendar months
National Institutes of Health
Contact: Can Varol, 31 Center Drive, Bldg. 31, Room 2A32, Bethesda, MD 20892-2425
The goal of this study is to develop biological and immunological test to identify AT1-AA as a pre-symptomatic risk factor for PE and to develop therapeutic strategies to prevent or treat PE based on blocking the pathophysiological consequences of autoantibody-induced angiotensin receptor activation.
Specific Aims: 1) Develop pre-symptomatic testing based on early detection of angiotensin receptor activating autoantibodies (AT1-AAs) associated with preeclampsia; 2) Develop therapeutic strategies to prevent or treat preeclampsia based on blocking autoantibody-induced angiotensin receptor activation.

"Preeclampsia and Autoimmunity"
2 R01 HD034130 (Kellems) 02/15/08-01/31/13 3.6 calendar months
National Institutes of Health
Contact: Grace Poe, 6100 Executive Blvd., BG 6100 RM 8A17H, Rockville, MD 20852
The goal was to determine how angiotensin receptor agonistic autoantibodies cause preeclampsia.
Specific Aims: 1) Evaluate the potential contribution of AT_1-AAs to the pathophysiology of preeclampsia; 2) Examine therapeutic strategies based on blocking autoantibody-induced AT_1 receptor activation; 3) Determine the mechanism of AT_1-AA-induced AT1 receptor activation.
Role: Co-Investigator

CURRENT

"Metabolites, Sickle Cell Disease, and Novel Therapeutics"
1 R01 HL113574 (Xia) 02/01/13-01/31/17 2.4 calendar months
National Institutes of Health
Contact: Kevin Heath, 6701 Rockledge Dr., RKL2 BG RM 7165, Bethesda, MD 20817
The goal of the proposed research is to determine newly identified metabolites in sickle cell disease and develop new therapies for the disease.
Specific Aims: 1) Determine the molecular basis underlying A2BR-mediated induction of 2,3-DPG in erythrocytes; 2) Determine pathogenic mechanisms underlying elevated S1P-induced sickling, inflammation and progression; 3) Determine molecular mechanisms responsible for the elevation of S1P in SCD; 4) Determine if elevated Ado, S1P and 2,3-DPG are novel pathogenic biomarkers that correlate to disease severity and phenotypic variation.
Role: Co-Investigator
Overlap: No overlap with the proposed project.

PENDING

"Adenosine Signaling in Priapism and Erectile Dysfunction and Novel Therapies"
2 R01 DK083559 (Xia) 04/01/15-03/31/20 1.2 calendar months
National Institutes of Health
Contact: This proposal was submitted early July 2014. A grants officer has not been assigned.

The major goal of this study is to provide significant new insight regarding priapism and ED pathogenesis and in turn provide new therapeutic options for safe and effective treatment.
Specific Aims: 1) Determine molecular basis underlying priapism in ENT2-deficient mice and the importance of reduced penile ENT2 in priapism in SCD mice; 2) Define novel role of erythrocyte ADORA2B-mediated O2 release in normal erection, priapism and ED; 3) Conduct translational studies to determine if circulating Ado and 2,3 levels of these pathogenic metabolites are associated with the phenotypic variation of priapism in SCD patients.
Role: Co-Investigator
Overlap: No overlap with the proposed project.

PREVIOUS/CURRENT/PENDING SUPPORT

Subudhi, A.

PREVIOUS (ending within the last 5 years)

AltitudeOmics: The Basic Biology of Human Acclimatization To High Altitude

W81XWH-11-2-0040 (Roach, PI) 01/2011-6/2014 (publications are still ongoing)
DMRDP
Contact: Jason Ghannadian, Telemedicine and Advanced Technology Research Center (TATRC)
Bldg. 1054 Patchel Street Fort Detrick, Maryland 21702-5012. (301) 619-0235

This project aims at advancing high-altitude medical research by discovering the basic molecular mechanisms of acclimatization that protect soldiers from high altitude illness.

No overlap with the proposed project.

CURRENT

"Prediction of acute mountain sickness using a blood-based test"
W81XWH-11-2-0034 (Roach, PI) 12/2010-12/2015 1.5 calendar
DMRDP
Contact: Jason Ghannadian, Telemedicine and Advanced Technology Research Center (TATRC)
Bldg. 1054 Patchel Street Fort Detrick, Maryland 21702-5012. (301) 619-0235

This project aims at developing a rapid, cost-effective, pre-ascent screening test to predict individual risk of acute mountain sickness (AMS) for military use.

No overlap with the proposed project.

"Combinational Drug Screening to Identify Strategies That Enhance Ground Troop Readiness at Altitude"
Contract # N66001-10-C-2134 (Irwin Pi, Roach Co-PI) 02/11-05/2015 1.5 calendar
DARPA
Contact: Shannon Kasa, SSC P Code 71510, San Diego, CA 92152 (619) 553-3889
The advancement of high-altitude medical research by discovering novel preventive measures for acute mountain sickness.

No overlap with the proposed project.

PENDING

"Three New Ideas to Protect the Special Forces From the Stress of High Altitude"
W81XWH-USSOCOM-BAA-14-1 (Roach, PI) 3 calendar
USSOCOM
This project will test the efficacy of three compounds we believe will prevent acute mountain sickness (AMS) and optimize performance of special operations forces (SOF) at operationally relevant altitudes of 10,000 to 13,000 feet. To achieve this objective we will select 60 men living at sea level who meet or exceed basic physical fitness requirements for SOF. These subjects will be assigned to one of four treatment groups (placebo, quercetin, nifedipine+ methazolamide, or metformin) and transported to high altitude to test the efficacy of the treatments on symptoms of acute mountain sickness (AMS) and physical and cognitive performance over a three-day period.

Overlap: some minor apparent overlap in that the same drug is tested. However, the aims of both projects are different in since this project exclusively analyzes performance whereas the current proposal analyzes oxygen transport.

PREVIOUS/CURRENT/PENDING SUPPORT

Zhang, Y.

PREVIOUS (ending within the last 5 years)
None

CURRENT

"Hypoxic Adenosine Responses"
Core C: Metabolite Core
1 P01 HL114457 (Blackburn) 06/01/13-05/31/18 1.2 calendar months
NIH
Contact: John Bucheimer, 6701 Rockledge Dr., RKL2 BG RM 7136, Bethesda, MD 20817
The purpose of this core is to quantify adenosine levels in samples gathered in all Projects to insure quality of results and in so doing contribute to the overall success of this PPG.
Specific Aims: 1) To quantify adenosine and its metabolites as part of their studies to understand the hypoxic adenosine response.
Role: Core C Leader
Overlap: No overlap with the proposed project.

PENDING
None

PREVIOUS/CURRENT/PENDING SUPPORT

BLACKBURN, M.R.

PREVIOUS (ending within the last 5 years)

"Role of A2B Adenosine Receptor and its Antagonist (GS-6201 and GS-6202) in Pulmonary Hypertension"
PO 4500020593/GS-6201 (Blackburn) 02/20/04-08/30/14 0.24 calendar months
Gilead Sciences, Inc.
The goal of this project is to examine the contribution of A_{2B} adenosine receptor signaling in adenosine-mediated pulmonary hypertension in ADA-deficient mice and the bleomycin model using selective A_{2B} receptor antagonists provided by Gilead. These studies provided preclinical data that led to the FDA filing of CVT-6883 for the treatment of pulmonary fibrosis and pulmonary hypertension.
Overlap: No overlap with the proposed project.

"Mediators of Pulmonary Vasodilatation in Liver Disease"
R01DK056804 (Fallon) 08/01/10-07/31/14 0.6 calendar months
NIH
The long-term goal is to use an understanding of vascular dysfunction in HPS to develop medical therapies and as a paradigm for understanding the pathogenesis of other vascular complications of liver.
Role: Co-I
Overlap: No overlap with the proposed project.

CURRENT

"Adenosine Signaling and Lung Fibrosis"
R01HL070952 (Blackburn) 07/01/02-06/30/15 3.6 calendar months
NIH/NHLBI
The goals of this study are to 1) examine A2BR-dependent IL-6 production as a mechanism for promoting pulmonary fibrosis; 2) examine mechanisms by which IL-6 influences pulmonary fibrosis; and 3) examine A2BR-mediated IL-6 production, IL-6R shedding and STAT-3 activation in IPF patients.
Overlap: No overlap with the proposed project.

"Hypoxic Adenosine Responses"
P01HL114457 (Blackburn) 06/01/13-05/31/18 3.6 calendar months
NIH
The goal of this program project is to define the pathways by which adenosine generation and signaling is beneficial in acute lung and kidney injury and detrimental during chronic lung disease (fibrosis) and sickle cell disease.
Project 1. *Hypoxic Adenosine Responses in the Regulation of Lung Injury.* (Blackburn)
The goal of this project is to examine the mechanisms underlying the protective effects of adenosine during acute lung injury, the detrimental effects of adenosine in chronic lung disease and the continuum between the two. The focus will be on the promotion of barrier function by adenosine during acute lung injury and the role of adenosine-dependent alternatively activated macrophages during fibrotic disease stages.
Core A. *Administrative Core*. (Blackburn)
The goal of this core will be to provide the administrative infrastructure needed to promote interactions between component project of this PPG.
Role: Prog Director, Project 1 PI, and Core A Leader
Overlap: No overlap with the proposed project.

"Center for Clinical and Translational Sciences (CCTS)"
UL1TR000371 (McPherson) 06/27/12-05/31/17 1.2 calendar months
NIH/National Center for Advancing Translational Sciences

The goal of the CCTS is to move scientific and medical discoveries as fast as possible from the laboratory to the clinic and community, where they can improve the health of the American people. The CCTS trains researchers, provides research services, and works with its communities to learn their health concerns and spread health care information.
Role: Co-director of the TL1 component of the CTSA award
Overlap: No overlap with the proposed project.

"Translational Regulation in Bronchial Epithelial Cells"
R01AI077679 (Shyu) 07/25/11-06/30/15 0.6 calendar months
National Institutes of Health
The aims of this proposal are to determine whether a reduction in miR-26 and miR-16 abundance contributes to the persistent, elevated level of IL-6 observed in asthmatic primary HBE cells; to define the role of a group of miRNAs that are significantly down-regulated in asthmatic primary HBE cells in controlling the activity of translation machinery in bronchial epithelial cells; and to determine whether a reduction in P-bodies is a hallmark of activated bronchial epithelial cells, and how alteration of P-body assembly and disassembly influences the inflammatory response in bronchial epithelial cells.
Role: Co-PI
Overlap: No overlap with the proposed project.

"Metabolites, Sickle Cell Disease, and Novel Therapeutics"
R01HL113574 (Xia) 02/01/13-01/31/17 0.6 calendar months
NIH/NHLBI
The goal of the proposed research is the development of novel approaches to ameliorate erythrocyte sickling in individuals with sickle cell disease (SCD).
Role: Co-I
Overlap: No overlap with the proposed project.

"Cadherin-11 Regulation of Fibrosis through Modulation of Epithelial-to-Mesenchymal Transition: Implications for Pulmonary Fibrosis in Scleroderma"
PR110864 (Agarwal) 09/30/12-09/29/15 0.6 calendar months
DOD/Baylor College of Medicine
The aims of this proposal are to determine the contribution of cadherin-11 to process of epithelial-to-mesenchymal transition in airway epithelial cells, to investigate the expression of cadherin-11 on alveolar macrophages and the cadherin-11 dependent regulation of TGF-beta production by alveolar macrophages and to determine if cadherin-11 is a key mediator of fibrosis in the intraperitoneal bleomycin model of pulmonary fibrosis and if cadherin-11 modulates epithelial-to-mesenchymal transition in vivo during the development of pulmonary fibrosis.
Role: Subaward Co-I
Overlap: No overlap with the proposed project.

PENDING
"Mechanisms of alveolar type II epithelial cell dysfunction in liver disease"
Pending # (Zhang) 04/01/15-03/31/20 0.24 calendar months
NIH
The goal of this project is to explore the mechanisms underlying the development of pulmonary complications in liver disease and focusing on vascular alterations including endothelial cells and monocytes.
Role: Co-I
Overlap: No overlap with the proposed project.

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2015 *** End Date:** 07-31-2016 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Yang		Xia	PhD	PD/PI		2.4					
2.	Dr.	Rodney	E.	Kellems	PhD	Collaborator		1.2					
3.		Yujin		Zhang		Collaborator		4.8					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
1	Graduate Students	12			29,000.00	6,670.00	35,670.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**35,670.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**127,722.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2015 *** End Date:** 07-31-2016 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,100.00
2. Foreign Travel Costs	
Total Travel Cost	**5,100.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2015 *** End Date:** 07-31-2016 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	45,000.00
2. Publication Costs	5,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shared Equipment Maintenance Fees & Genotyping Service Fee	9,700.00
9. Animal Housing and Maintenance Fees	46,672.00
10. Graduate Student Tuition and Fees	4,967.00
Total Other Direct Costs	**111,339.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**244,161.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Research On_Campus	54	239,194.00	129,165.00
		Total Indirect Costs	**129,165.00**

Cognizant Federal Agency DHHS, Arif Karim, (214) 767-3261

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**373,326.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: BudgetJustification1015597631.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2016 *** End Date:** 07-31-2017 **Budget Period: 2**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Yang		Xia	PhD	PD/PI							
2.	Dr.	Rodney	E.	Kellems	PhD	Collaborator		1.2					
3.		Yujin		Zhang		Collaborator		4.8					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **94,814.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
1	Graduate Students	12			29,870.00	6,870.00	36,740.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**36,740.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**131,554.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2016 *** End Date:** 07-31-2017 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,300.00
2. Foreign Travel Costs	
Total Travel Cost	**3,300.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2016 *** End Date:** 07-31-2017 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	44,290.00
2. Publication Costs	5,150.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shared Equipment Maintenance Fees & Genotyping Service Fee	9,991.00
9. Animal Housing and Maintenance Fees	48,072.00
10. Graduate Student Tuition and Fees	5,116.00
Total Other Direct Costs	**112,619.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**247,473.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Research On_Campus	54	242,357.00	130,873.00
		Total Indirect Costs	**130,873.00**

Cognizant Federal Agency DHHS, Arif Karim, (214) 767-3261

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**378,346.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: BudgetJustification1015597631.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2017 *** End Date:** 07-31-2018 **Budget Period: 3**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Yang		Xia	PhD	PD/PI		2.4					
2.	Dr.	Rodney	E.	Kellems	PhD	Collaborator		1.2					
3.		Yujin		Zhang		Collaborator		4.8					

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **97,659.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
1	Graduate Students	12			30,766.00	7,076.00	37,842.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**37,842.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**135,501.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2017 *** End Date:** 07-31-2018 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,300.00
2. Foreign Travel Costs	
Total Travel Cost	**3,300.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant/Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 800771594

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of Texas Health Science Center at Houston

*** Start Date:** 08-01-2017 *** End Date:** 07-31-2018 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	45,620.00
2. Publication Costs	5,305.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Shared Equipment Maintenance Fees & Genotyping Service Fee	10,291.00
9. Animal Housing and Maintenance Fees	49,514.00
10. Graduate Student Tuition and Fees	5,269.00
Total Other Direct Costs	**115,999.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**254,800.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Research On_Campus	54	249,531.00	134,747.00
		Total Indirect Costs	**134,747.00**

Cognizant Federal Agency DHHS, Arif Karim, (214) 767-3261
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**389,547.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: BudgetJustification1015597631.pdf Mime Type: application/pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		**284,525.00**
Section B, Other Personnel		**110,252.00**
Total Number Other Personnel	3	
Total Salary, Wages and Fringe Benefits (A+B)		**394,777.00**
Section C, Equipment		
Section D, Travel		**11,700.00**
1. Domestic	11,700.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**339,957.00**
1. Materials and Supplies	134,910.00	
2. Publication Costs	15,455.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	29,982.00	
9. Other 2	144,258.00	
10. Other 3	15,352.00	
Section G, Direct Costs (A thru F)		**746,434.00**
Section H, Indirect Costs		**394,785.00**
Section I, Total Direct and Indirect Costs (G + H)		**1,141,219.00**
Section J, Fee		

BUDGET JUSTIFICATION

PERSONNEL (Initial - $127,722; Total - $394,777)

Senior/Key Personnel

Yang Xia, M.D., Ph.D., Principal Investigator (2.4 calendar months effort) is a Professor in the Department of Biochemistry and Molecular Biology at the University of Texas Medical School at Houston. She is a highly trained biochemist/molecular biologist who has received M.D./Ph.D. training with extensive experience in translational studies. Dr. Xia initiated, oversees and coordinates all the proposed research activities. Dr. Xia's lab is the first to discover that increased adenosine contributes to erythrocyte sickling in sickle cell disease by induction of 2,3-BPG. More recently, he has collaborated with Dr. Robert Roach in high altitude hypoxia human studies and further demonstrated that circulating adenosine is also significantly elevated in the high altitude and its elevation is correlated to elevated 2,3-BPG levels. Dr. Xia's laboratory has extensive experience in preclinical studies proposed in this application. Dr. Xia has and will continue to participate in all experimental protocols, as well as supervise work at the bench. She will be directly involved in data analysis, the writing of manuscripts for publication, as well as progress reports. She also provides all the laboratory space, basic equipment, and reagents for nearly all the experiments proposed.

Rodney E. Kellems, Ph.D., Collaborator (1.2 calendar months) is Professor and Chairman of the Department of Biochemistry and Molecular Biology at The University of Texas Health Science Center at Houston. He received his Ph.D. training at Princeton University and postdoctoral training at Stanford University. Dr. Kellems has many years of experience in biochemistry, molecular biology, genetics and immunology. He and Dr. Xia have collaborated for more than ten years and their combined efforts have led to many key publications including JCI and Nature Medicine. Dr. Kellems is extremely excited about the current proposal and will continuously provide his expertise in adenosine signaling and mouse genetics to the proposed studies. He will assist Dr. Xia to accomplish the goals to translate our current finding to novel therapies for COPD.

Yujin Zhang, M.D., Ph.D., Collaborator (4.8 calendar months effort) is a well-trained molecular and cellular biologist with extensive experience with transgenic and genetically deficient mice handling and maintenance. He received medical training in hematology in China before earning his PhD degree in the US. He has assisted Dr. Xia for seven years. Most of the preliminary data obtained from sickle cell disease humans and transgenic mice experiments were generated by him. Some of these studies resulted in our recent paper published in Nature Medicine (Zhang, et al 2011) and JCI (Zhang, et al, 2014). More recently, he has identified that equilibrative nucleoside transporter is a key factor controlling circulating adenosine under hypoxia. For this project, Dr. Zhang will contribute his effort to conduct preclinical studies to determine if dipyradomole, a FDA approved drug, is a safe and effective drug to treat and prevent lung damage and progression to COPD as proposed in AIM I.

Other Personnel

Hong Liu, Graduate Research Assistant (12 calendar months effort), is an PhD student who chose to conduct his thesis research in the Xia laboratory with Dr. Xia as his major thesis advisor. Within a short two year period in Dr. Xia's laboratory, Hong has become very familiar with mouse handling, molecular biology and histological studies. Intriguingly, with the involvement in high altitude hypoxia studies, he has discovered that AMPK is a key signaling network regulating erythrocyte O2 release to peripheral tissues by inducing 2,3-BPG. This study setup a strong foundation for proposed preclinical studies and pilot human studies in AIM II and III. He will continue his effort to conduct preclinical studies to test the efficacy of metformin, a FDA approved drug, in treatment and prevention of COPD in AIM II.

Salary support for all personnel is equal to the level of effort contributed to the project. All increases in salaries, excluding the initial year, are calculated at 3% per year. Actual fringe benefits are calculated based on total compensation rates.

Other Significant Contributor(s)

Michael Blackburn, Ph.D., Other Significant Contributor (no salary or effort requested) is a Professor in the Department of Biochemistry and Molecular Biology at The University of Texas Health Science Center at Houston. Dr. Blackburn is an expert in the role of adenosine signaling in pulmonary disease and will provide his expertise in adenosine signaling and pulmonary disease. Dr. Blackburn and Dr. Xia have collaborated for many years and co-authored papers including those appearing in Nature Medicine, JCI, FASEB J and JSM. Dr. Blackburn has an extensive experience in measuring pulmonary inflammation in mice. He will continuously provide the PI with his expertise in pulmonary damage analysis in mice.

EQUIPMENT (Initial - $0; Total - $0)
Funds are not requested for this category.

TRAVEL (Initial - $5,100; Total - $11,700)
Funds ($1,800) are requested for the PI to attend one national scientific meeting per project year to present findings from this study to the greater scientific community. In addition, the PI will travel to the University of Colorado Denver once per project year for a collaborative meeting with Dr. Roach (Partnering PI) to review the progress on the project and coordinate future efforts ($1,500/year). Per the program announcement, funds ($1,800) are also requested during the first project year for the PI to disseminate project results at one DoD-sponsored meeting to be specified by the CDMRP during the award performance period. Costs include ground travel, lodging, meals, and registration. The total estimated cost for each year is as follows: 1st year - $5,100; 2nd year - $3,300 and 3rd year - $3,300.

OTHER DIRECT COSTS (Initial - $111,339; Total - $339,957)

Materials and Supplies:

Standard lab supplies: Funds are requested for glassware, plasticware, microcentrifuge tubes, and standard lab chemicals. These materials and supplies will be used for the generation, usage and storage of reagents used in the various molecular and cell biology experiemnts proposed. In addition, supplies will be needed for personnel safety, the maintenance of the lab environment and the documentation of results (gloves, diapers, paper towels, biohazard supplies, lab notebooks etc.). These supplies are essential for the sucessful completion of the proposed experiments. Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $2,000; 2nd year - $2,060 and 3rd year - $2,122.

Microarrays and metabolic profiling and quantification: This study will require approximately 20 microarrays per year (~$250/microarray) and 20 metabolomic screenings and further quantifications by RT-PCR and HPLC and Tendom double mass spectural analysis by Metabolon. Inc per year (~350/screening). Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $12,000; 2nd year - $12,360 and 3rd year - $12,731.

Molecular biology studies: Funds are requested for molecular biology reagents/kits and real time Q-PCR supplies. This includes specialized reagents for the isolation of DNA, RNA and the conduction of PCR, real time quantitative PCR. Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover

anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $4,500; 2nd year - $4,635 and 3rd year - $4,774.

Protein analysis: Funds are requested for the purchase of Western blot supplies (~$2,000/year) , ELISA supplies (~3,500/year), and flow cytometry supplies (~$2,000/year) including antibodies and associated substrates and materials. The total estimated cost for each year is as follows: 1st year - $7,500; 2nd year - $7,725 and 3rd year - $7,957.

Histological and immunohistological analysis: Funds are requested for histological supplies including alcohols and other materials for tissue embedding, sectioning and stains; as well as, antibodies and kits necessary for the analysis of protein experssion in tissue sections. Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $4,500; 2nd year - $4,635 and 3rd year - $4,774.

Animal Associated Supplies: Funds are requested each year for the purchase (~$4,500/year) and shipping costs (~$500/year) of approximately 200 wild type C57/Bl6 mice from Jackson Laboratories for use as controls in experiments. In addition, mefformin, dipyridomole, and other related reagents (~$3,000/ year) will be needed for the mice experiments every year. This project will also require ~$4,500/year for performing genotyping of genetic mice. For the first project year only, funds are requested to purchase a large hypoxia chamber ($2,000). Costs (excluding the purchase of the hypoxia chamber) are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $14,500; 2nd year - $12,875 and 3rd year - $13,262.

Publication Costs:

Funds are requested during all project years for publication costs such as manuscript submission fees (~$75), color charges (~$675-$800), and page charges (~$600) for 4-6 papers published in such journals as Nature Medicine, JCI and other related journals. Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $5,000; 2nd year - $5,150 and 3rd year - $5,305.

Shared Equipment Maintenance Fees:

The Biochemistry and Molecular Biology Department has several common that are available for shared use by PIs. These equipment are maintained through joint contributions based on usage time. For this project, the centrifuges (~$500/year), scintillation counters (~$800/year), autoclaves (~$1000/year) spectrophotometers (~$500/year), HPLC (~$500/year), Confocol (~$700/year), and flow cytometry (~$1000/year) common equipment will be utilized on a day to day basis and are critical to the success of the experiments proposed. Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $5,000; 2nd year - $5,150 and 3rd year - $5,305.

Genotyping Service Fee: Some of the proposed genetically manipulated mice cannot be analyzed by the PI's laboratory. Therefore, the tail of mice will be prepared by the project team and shipped to Genetype Inc. for analysis (~$4,700/year). Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $4,700; 2nd year - $4,841 and 3rd year - $4,986.

Animal Housing and Maintenance Fees: Funds are requested each year for the housing and maintenance fees of the proposed mice. Animals are housed and maintained by the Center for Laboratory Animal Medicine and Care at The University of Texas Health Science Center at Houston Center. Care days are estimated at $0.84/cage /day for 135 cages of mice. These cages will house C57/Bl6 mice, EpoR-Cre mice, Adora2bf/f-Epo-Cre mice, ENT1 and ENT2-deficient mice. Surgery costs include veterinary technician time ($37.50/hour), the rental of the rodent anesthetic machine ($6.40/hour), and isoflurane ($27.50 per 250mL). Other proposed

mouse expenses include complete blood count tests ($7.60/mouse) for all project years. These estimated expenses are critical to the overall success of this project. Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $46,672; 2nd year - $48,072 and 3rd year - $49,515.

Graduate Student Tuition and Fees: Tuition and fees for 1 graduate student is requested each year. This is essential for the graduate student to accomplish his Ph.D training. Costs are escalated during the 2nd through the 3rd project years at 3% per year to cover anticipated inflation in prices. The total estimated cost for each year is as follows: 1st year - $4,967; 2nd year - $5,116; 3rd year - $5,269.

INDIRECT COSTS (Initial - $129,165; Total - $394,785)

Funds for indirect costs are requested. Indirect costs are calculated based on 54% of the total modified direct costs per the institution's F&A agreement dated 07/17/14. The total estimated cost for each year is as follows: 1st year - $129,165; 2nd year - $130,873 and 3rd year - $134,747.

Attachments Form

Instructions: On this form, you will attach the various files that make up your grant application. Please consult with the appropriate Agency Guidelines for more information about each needed file. Please remember that any files you attach must be in the document format and named as specified in the Guidelines.

Important: Please attach your files in the proper sequence. See the appropriate Agency Guidelines for details.

1) Please attach Attachment 1	ProjectNarrative1015647051.pdf	Mime Type: application/pdf
2) Please attach Attachment 2	Support1015647024.pdf	Mime Type: application/pdf
3) Please attach Attachment 3	TechAbs1015646861.pdf	Mime Type: application/pdf
4) Please attach Attachment 4	LayAbs1015646873.pdf	Mime Type: application/pdf
5) Please attach Attachment 5	SOW1015646865.pdf	Mime Type: application/pdf
6) Please attach Attachment 6	Impact1015646862.pdf	Mime Type: application/pdf
7) Please attach Attachment 7	MilRel1015646863.pdf	Mime Type: application/pdf
8) Please attach Attachment 8	Partnership1015646866.pdf	Mime Type: application/pdf
9) Please attach Attachment 9		
10) Please attach Attachment 10		
11) Please attach Attachment 11		
12) Please attach Attachment 12		
13) Please attach Attachment 13		
14) Please attach Attachment 14		
15) Please attach Attachment 15		

A. Background. 1. Research Area of Respiratory Health. This proposed project tests two FDA-approved drugs for their effectiveness in treating hypoxia in respiratory disease by elevating tissue oxygen (O_2) delivery. Hypoxia is a major contributor to and consequence of many respiratory and cardiovascular diseases. For example, hypoxia is a major component of chronic obstructive pulmonary disease (COPD), a clinically devastating disease of increasing prevalence, occurring more in military veterans than in the general population.[1-3] And many COPD patients, even those with mild disease, regularly suffer additional hypoxia during sleep.[4] Healthy troops can also be exposed to hypoxia through deployment to high altitudes (e.g. to mountainous regions of Afghanistan or in CONUS at Fort Carson, Colorado with >6000 ft average altitude of residence). Thus, tissue O_2 delivery is limited for DoD personnel exposed to high-altitude hypoxia, and veterans with COPD. Limitations in tissue O_2 delivery decrease overall well-being and cognitive function, and directly cause poor physical performance (even walking ability is limited).[5]

Elevating tissue O_2 delivery will reverse the limitations to physical and cognitive performance. Tissue O_2 delivery can be improved by increasing O_2 uptake at the lungs and/or by increasing offloading of O_2 at the tissue. O_2 uptake can be raised by breathing supplemental oxygen or descending to a lower altitude. Alternatively, elevating tissue O_2 delivery can be achieved by changing the bond between O_2 and hemoglobin (Hb) to favor tissue O_2 unloading. Two drugs (metformin and dipyridamole) are approved by the FDA for other medical applications, and have been shown by our team to promote tissue O_2 delivery in mice. We have also shown for the first time that in hypoxic but otherwise healthy humans, the mechanisms to improve tissue O_2 delivery share many common features with the hypoxic mouse models. Adenosine and 2,3-BPG are the key components of this signaling pathway where elevated adenosine-driven elevation in 2,3-DPG levels leads to more O_2 unloading at the tissue. Adenosine is a purine nucleoside with many important biological properties,[6] and 2,3-BPG is an erythrocyte specific metabolite that induces O_2 release from Hb.[7] Determining the molecular and metabolic pathways that metformin and dipyridamole use to elevate 2,3-DPG in hypoxia, and their effectiveness in preventing and treating hypoxia-induced lung damage are the goals of Specific Aims I and II (see **Fig. 1**). Testing the translation of these ideas by making the first steps to studies in patients by using metformin and dipyridamole in hypoxic but otherwise healthy humans is the goal of Specific Aim III (see **Fig. 1**). Now we briefly review the preliminary data supporting these ideas.

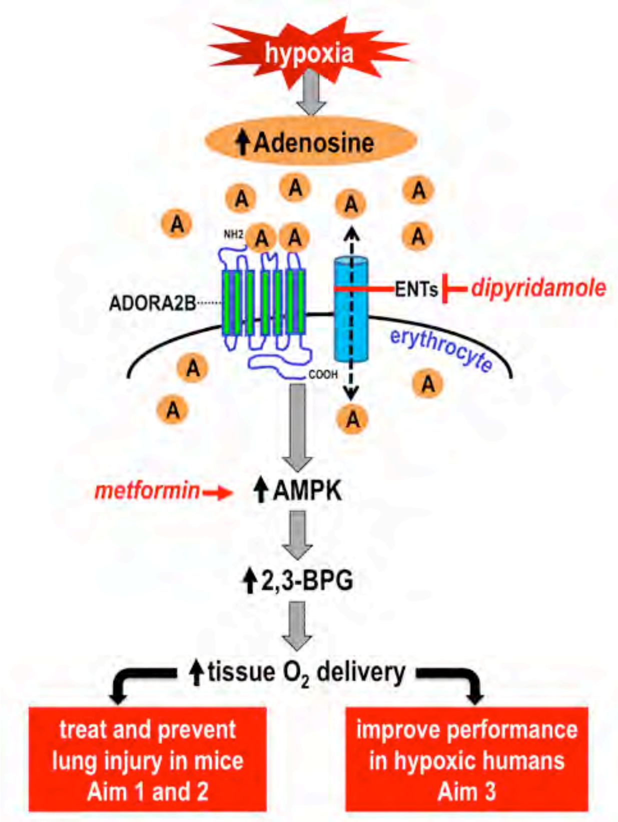

Fig. 1. Overview of the hypoxia signaling pathway acting through elevated adenosine (A) leading to elevated 2,3-BPG that drives an increase in tissue O2 delivery forming the basis for the experiments proposed for Aim 1 to 3

2. Preliminary Data.

i) Discovery that elevated circulating adenosine levels lead to raised 2,3-BPG concentrations thereby triggering O_2 release from red blood cells.[6] To identify the potential metabolites contributing to hypoxia-mediated disease development, we conducted a non-biased high throughput metabolomic screen using a well-accepted humanized mouse model of sickle cell disease, a condition characterized by chronic hypoxia. Among 7000 small metabolites screened, adenosine and 2,3-BPG were identified to be elevated in the circulation of both humans and mice with sickle cell disease. Although the discovery was made in sickle cell disease, subsequent work has shown universality to this hypoxia responsive pathway in various cell and animal preparations as well as in humans.

ii) Discovery that ADORA2B is required for hypoxia-induced 2,3-BPG production and subsequent elevated O_2 release (Fig. 2).[6,8] To determine the role of erythrocyte ADOR-

A2B on normal erythrocyte physiology, we have recently generated a novel mouse line with erythrocyte specific deletion of ADORA2B by crossing floxed ADORA2B mice with mice expressing Cre recombinase under the control of the erythropoietin receptor gene regulatory elements (EpoR).[9] These mice function well under normoxia. However, following 10% hypoxia for a week, we found that genetic deletion of erythrocyte ADORA2B attenuated adenosine-induced 2,3-BPG and O_2 release (**Fig. 3A-C**). In contrast circulating adenosine, erythrocyte 2,3-BPG and O_2 release are significantly induced in the control ADORA2B$^{f/f}$ mice (**Fig. 3A-C**). Thus, these findings provide the first genetic evidence that erythrocyte ADORA2B is required for hypoxia-induced 2,3-BPG production and subsequent O_2 release. This novel mouse line provides an important genetic tool to further determine the molecular basis underlying erythrocyte ADORA2B in hypoxia-induced lung damage in Aim I.

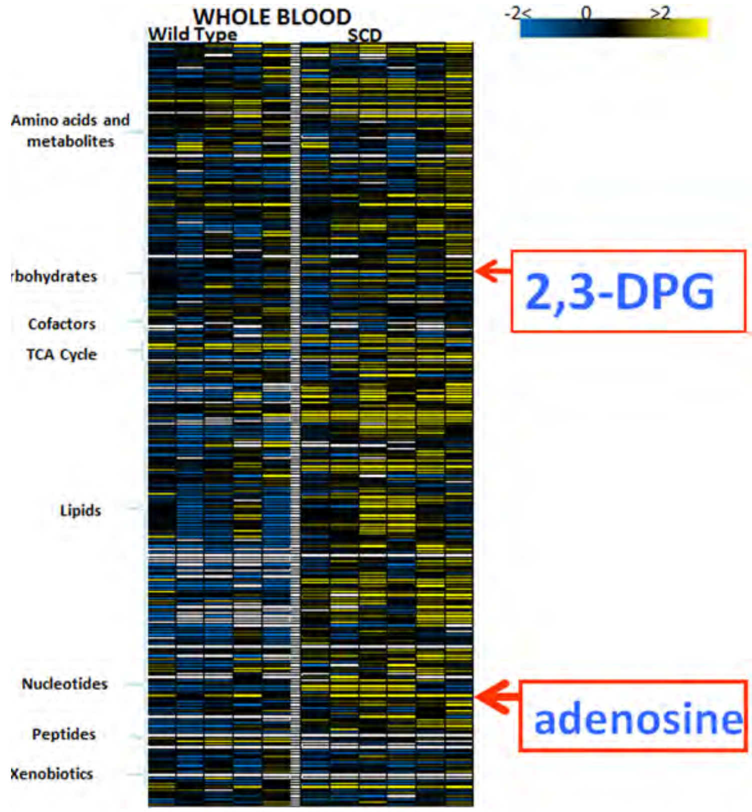

Fig. 2. Metabolomic profiling of whole blood of wild type (WT) and SCD Tg mice. Heat map showing that adenosine and 2,3-BPG, were increased in the whole blood of SCD Tg mice. n=6

iii) Discovery that AMP activated protein kinase (AMPK) functions downstream of ADORA2B to induce 2,3-BPG production and O_2 release from erythrocytes, suggesting AMPK as a novel target to enhance tissue O_2 delivery. Building on this work we next identified that AMPK, a well-known energy sensor, is a key molecule functioning downstream of ADORA2B underlying adenosine-induced erythrocyte 2,3-BPG production. Extending from this observation, we conducted *in vitro* experiments to assess if enhancing AMPK is sufficient to directly induce 2,3-BPG production and O_2 release in cultured mouse erythrocytes. Intriguingly, we found that two independent AMPK activators, AICAR and metformin, significantly increased 2,3-BPG production and O_2 release capacity in cultured erythrocytes (**Fig. 4A-B**). Our findings immediately suggest that metformin is likely a novel therapy for hypoxic lung diseases by enhancing O_2 release from erythrocytes to lungs to prevent hypoxia-induced pulmonary injury and progression to fibrosis. We will address this possibility in Aim I, and begin the process of translating these findings to human patients in Aim III.

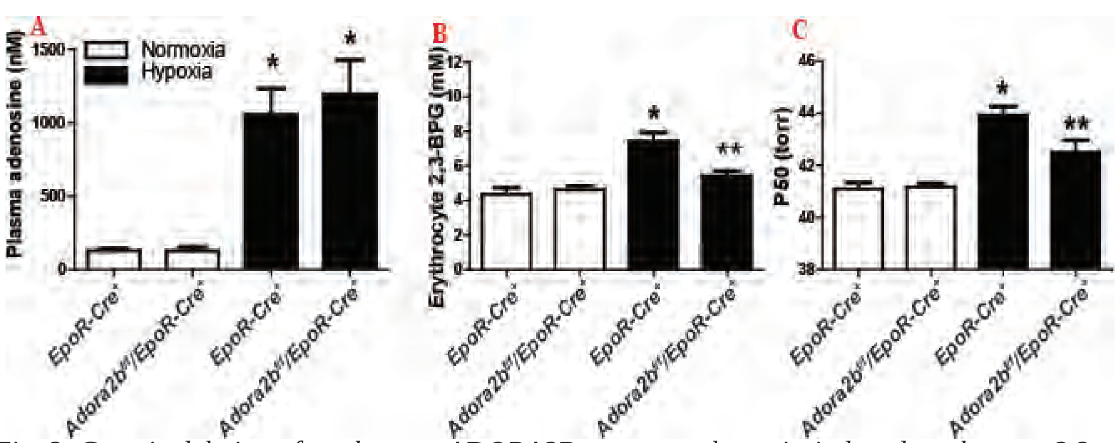

Fig. 3. Genetic deletion of erythrocyte ADORA2B attenuates hypoxia-induced erythrocyte 2,3-BPG (B) and O2 release (C) in mice. Ten week old mice were exposed under normoxia and 10% hypoxia for 1 week. At the end, mouse blood was collected. EpoR-Cre: control mice; Adora2bf/f-Epo-Cre: erythrocyte specific deletion of Adora2b in mice. N=10-15.

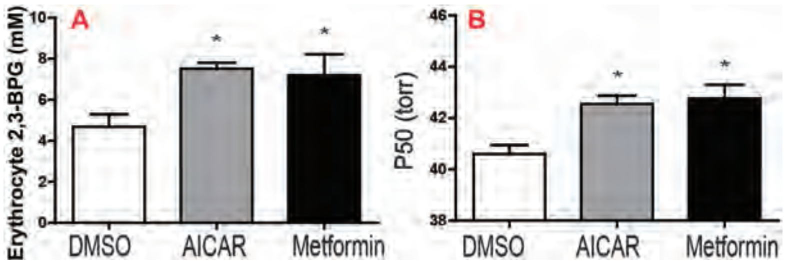

Fig. 4. (A-B) Both Metformin and AICAR directly induce 2,3-BPG levels and P50 in cultured erythrocytes.

iv) Discovery of critical role for equilibrative nucleoside transporters (ENTs) in 2,3-BPG production and O_2 release from normal erythrocytes, suggesting ENTs as another novel target to enhance tissue O_2 delivery. A critical pathway for regulating extracellular adenosine levels is the cellular uptake of adenosine through facilitated ENTs.[10,11] However, nothing is known about ENTs and erythrocyte physiology. To test this the role of ENTs in erythrocyte O_2 handling, we used dipyridamole, a drug that is a potent ENT inhibitor. We found that treatment with dipyridamole significantly increased circulating adenosine and enhanced erythrocyte 2,3-BPG production and O_2 releasing capacity in normal mice under normoxia **(Fig. 5A-C).** These findings suggest that inhibition of adenosine uptake by dipyridamole is likely a safe treatment for hypoxia by enhancing extracellular adenosine-induced 2,3-BPG production and O_2 release by erythrocytes. We will address this possibility in Aim II, and begin the process of translating these findings to human patients in Aim III.

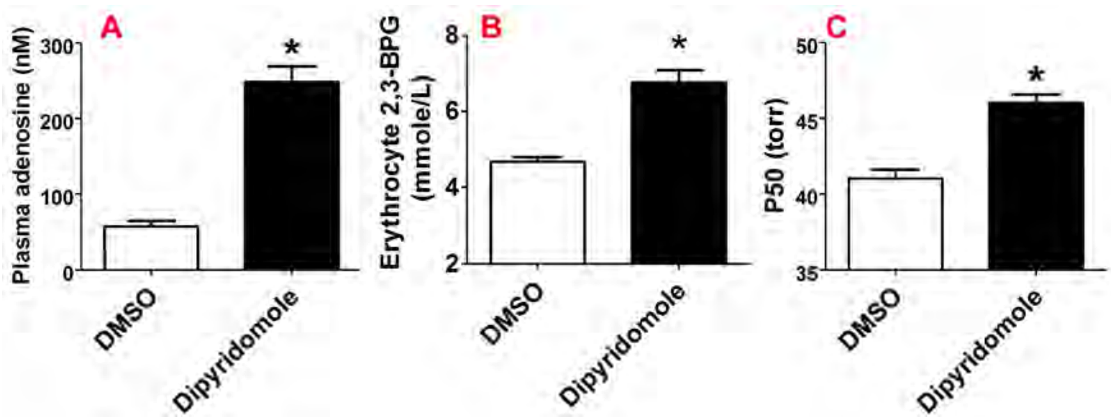

Fig.5. Inhibition of ENTs by dipyridamole induced plasma adenosine (A), erythrocyte 2,3-BPG (B) and P50 (C) in normal mice under normoxia

v) Discovery that the adenosine-2,3-BPG pathway described above in cell and animal studies exists and functions in a similar manner in hypoxic but otherwise healthy humans. To test for the existence of the adenosine-2,3-BPG-AMPK pathway in hypoxic but otherwise healthy humans, the partnering PIs on the present proposal collaborated on a DoD-funded study of the basic mechanisms of cellular adjustment to hypoxia by testing 21 healthy individuals exposed to hypoxia over several weeks.[12-19] We found that as the volunteers adjusted to hypoxia they experienced elevated blood oxygen levels (PaO_2, SaO_2 and CaO_2 all rose significantly by day 16), and they experienced improved exercise and cognitive performance. Accompanying these physiological changes were elevated adenosine and 2,3-BPG levels after 16 days of hypoxia. The elevated circulating adenosine levels significantly correlated with increased erythrocyte 2,3-BPG levels (**Fig. 6A-E**). In addition and similar to our mouse findings, we found that erythrocyte AMPK activity was significantly elevated and its elevation significantly correlated to levels of circulating adenosine and erythrocyte 2,3-BPG (**Fig. 7A-C**). And at 16 days when adenosine, 2,3-BPG levels and AMPK were at their highest levels, the P50, a measure of the partial pressure of O_2 in the blood that is a reliable indicator of Hb-O_2 binding affinity, was higher and significantly correlated to the 2,3-BPG levels. The means that tissue O_2 unloading was at its highest level when subjects were most well adjusted to hypoxia.

It is important to keep in mind what is new about these findings. Since the classic studies in the 1960's,[20-22] we have known that 2,3-BPG levels rise after long-term exposure to hypoxia. By discovering that adenosine and AMPK rise along with 2,3-BPG helped us identify the mechanism of this important adaptive response. With a clearer understanding of the mechanism we propose two innovative pharmacological strategies aimed at increasing 2,3 BPG concentrations in people who are acutely hypoxia, such as a soldier flying to high altitude or a patient who has not successfully counteracted hypoxia. We expect these pharmacological strategies will improve cognitive and physical performance in these groups. Taking the next step will examine if raising 2,3-BPG levels via AMPK with metformin or via ENTs with dipyridamole improves tissue O_2 delivery in vivo in hypoxic but otherwise healthy humans.

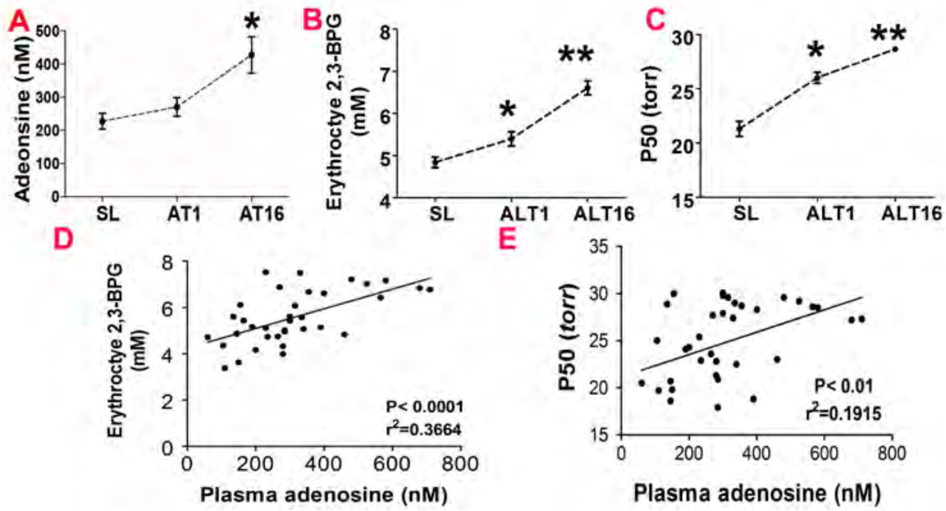

Fig. 6. High altitude hypoxia induces plasma adenosine (A), erythrocyte 2,3-BPG (B) and P50 (C) (O2 releasing capacity) in a time dependent manner and their levels correlated to each other (D-E). n=20

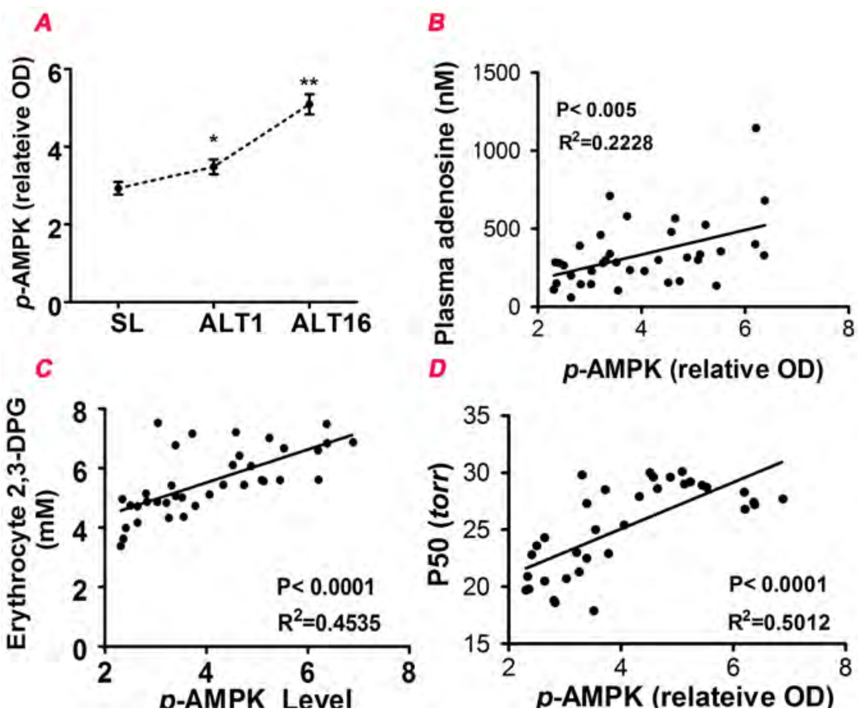

Fig. 7. High altitude hypoxia induces activation of AMPK p-AMPK,(A) and its level correlates to plasma adenosine (B), 2,3-BPG (B) and P50 (C) . n=20

B. Research Objective and Strategy. Our work described above leads to the compelling hypothesis that increasing extracellular adenosine by enhancing AMPK activation by metformin or by inhibition of ENTs by dipyridamole resulting in elevated levels of 2,3-BPG are promising new approaches to improving tissue O_2 delivery in patients with respiratory disease and in hypoxic healthy subjects (**Fig. 1**). In the proposed new studies we will extend our current discoveries by conducting preclinical studies to determine the therapeutic effects of metformin and dipyridamole in two independent experimental models of lung injury (Aim I and II) and conduct mechanistic studies by identifying additional factors and signaling pathways involved in erythrocyte ADORA2B-mediated O_2 release in response to hypoxia using our newly developed erythrocyte mouse line (Aim I). We also propose human pilot studies to test if metformin and dipyridamole improve tissue O_2 delivery for counteracting hypoxia in Aim III. In this Partnering PI grant proposal, Aims I and II are proposed by the Xia team from Houston, and Aim III is proposed by the Roach team from Denver. Dr. Xia's team is the first to discover that circulating adenosine causes an elevation in 2,3-BPG levels and thus boosts oxygen delivery. Extending this finding, Dr. Xia and Dr. Roach combined their efforts to further discover that hypoxia-induced adenosine is likely beneficial to trigger O_2 release to enhance human adjustment to hypoxia. Thus, by collaborating on this proposal the partnering PIs, with their multidisciplinary and interdisciplinary

expertise, can make significant contributions to understanding how to improve tissue O_2 delivery in hypoxemic humans, whether the root cause is respiratory disease or other environmental causes of hypoxia. It is our hope that within the next 3 years, our proposed studies will provide us new insight into the advanced treatment options for hypoxia-linked respiratory diseases, including COPD and any other humans experiencing hypoxia.

C. Specific Aims. To accomplish our objective, three specific aims are proposed:

> **Aim I.** Conduct preclinical studies to determine if metformin can reduce hypoxia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling in hypoxia-induced lung damage and disease progression.
>
> **Aim II.** Conduct preclinical studies to determine the therapeutic effects of in hypoxia-induced lung damage and disease progression by inducing circulating adenosine, erythrocyte 2,3-BPG and O_2 release.
>
> **Aim III.** To use metformin and dipyridamole to elevate oxygen delivery in healthy humans experiencing hypoxia to examine the consequences on physical and cognitive performance, and to choose the most effective approach to manipulating oxygen delivery for future studies on COPD patients.

Aim I. Conduct preclinical studies to determine if metformin can reduce hypoxia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling in hypoxia-induced lung damage and disease progression.

Q1. Can metformin reduce hypoxia-induced lung damage in WT and AdoRA2B$^{f/f}$-Epo-Cre mice?

Rationale. Our preliminary studies showed that the activation of AMPK is essential to stimulate 2,3-BPG production and promote O_2 release from Hb in erythrocytes via ADORA2B signaling. Using hypoxia probe to assess tissue hypoxia levels in lung tissues, severe pulmonary hypoxia with enlarged airway spaces and vascular damage was observed in AdoRA2B$^{f/f}$/E*poR-Cre*$^{+}$ mice, while only a slight hypoxia signal was present in the lung of E*poR-Cre*$^{+}$ mice (**Fig. 8A**). Additionally, we found that hypoxia-induced erythrocyte AMPK activity was significantly reduced in *AdoRA2B$^{f/f}$-EpoR-Cre* compared to *EpoR-Cre mice (***Fig. 8B***)*. Thus, these preliminary studies implicate that erythrocyte ADORA2B-mediated AMPK activation is essential for hypoxia-induced adenosine-mediated 2,3-BPG induction and O_2 release from erythrocyte to prevent hypoxia-induced lung damage. Although the molecular basis of metformin's action is not fully understood, it is well known to activate AMPK. Thus, we propose to test *i) if enhancing AMPK signaling by metformin can sufficiently rescue hypoxia-induced lung damage in AdoRA2B$^{f/f}$-EpoR-Cre mice; and ii) if metformin is capable of preventing or treating COPD in normal wild type (WT) mice by reducing hypoxia.*

Approach. We will use two independent animal models of lung injury coupled with our newly developed erythrocyte-specific ADORA2B knockouts to investigate the general efficacy of metformin in hypoxia-induced lung damage and disease progression (**Fig. 9**). As shown in **Fig. 8A**, systemic hypoxia in wild mice directly induced mild lung vascular remolding and pulmonary ede-

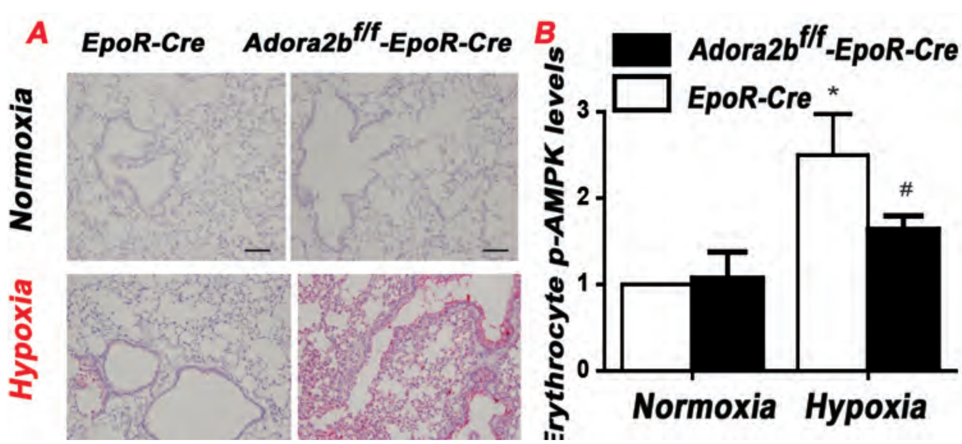

Fig.8. Increased hypoxia levels and reduced p-AMPK levels in Adora2bf/f/EpoR-Cre+ mice compared to EpoR-Cre mice.

ma. Without erythrocyte ADORA2B, hypoxia-induced pulmonary vascular damage and pulmonary edema were much severe compared to the control EpoR-Cre mice. This study indicates that systemic hypoxia induces acute pulmonary damage. Additionally, intra-tracheal injection of bleomycin is another well-established experimental model for lung damage.[23,24] It is likely, without interference, hypoxia induced by bleomycin-induced lung tissue damage will progress to pulmonary fibrosis. Notably, our recent published studies showed that pulmonary adenosine is induced in this acute bleomycin-induced lung injury model by intra-tracheal (IT) injection of bleomycin.[23,24] In this study, we showed that elevated pulmonary adenosine protects bleomycin-induced acute lung damage and prevents progression to fibrosis.[24] However, the beneficial role of elevated adenosine-induced erythrocyte ADORA2B-AMPK activation in bleomycin-induced acute lung damage and progression to lung fibrosis by promoting O_2 release to reduced hypoxia has not addressed in this model prior to our recent discovery. Thus, we will test the efficacy of metformin in these two pulmonary injury models. Briefly, we will expose WT mice, *EpoR-Cre* (control) and $AdoRA2B^{f/f}$-*EpoR-Cre* mice to different degrees of hypoxia (range from 6-10% O_2) for different periods of time (24hr-2 weeks). Alternatively, we will also challenge those mice with IT injection of bleomycin to quickly induce acute lung damage and hypoxia (**Fig. 9**). Thus, 1) *to assess if metformin pretreatment can prevent hypoxia-induced lung damage (i.e. prevention)*, mice will be pretreated with different dosages of metformin[25] prior to exposure to hypoxia or IT injection of bleomycin; 2) *to test if metformin can reduce pulmonary damage and slow disease progression (treatment)*, we will start to treat mice after 24 hours at 8% hypoxia challenge or 24 hours after bleomycin IT injection with different dosages of metformin. At different time points, prior to sacrifice, we will collect bronchoalveolar (BAL) fluid to quantify alveolar damage by measuring albumin leakage in BAL and inflammation by measuring immune cells in BAL as before.[26] Once the mice are sacrificed, lungs will be collected and the ratio of wet weights to dry weights will be used to quantify the degree of lung edema.[26] Moreover, we will monitor hypoxic levels in the lung by hypoxia probe.[27] Tissue damage will be analyzed by histological analysis (HE and Trichrome staining). Pulmonary neutrophil sequestration will be quantified using a MPO assay and lung inflammation will be measured by multiple cytokine-ELISA array kit. Vascular leakage will be measured by Evans blue staining.[28] Finally, we will quantify plasma adenosine levels, erythrocyte 2,3-BPG levels and P50.

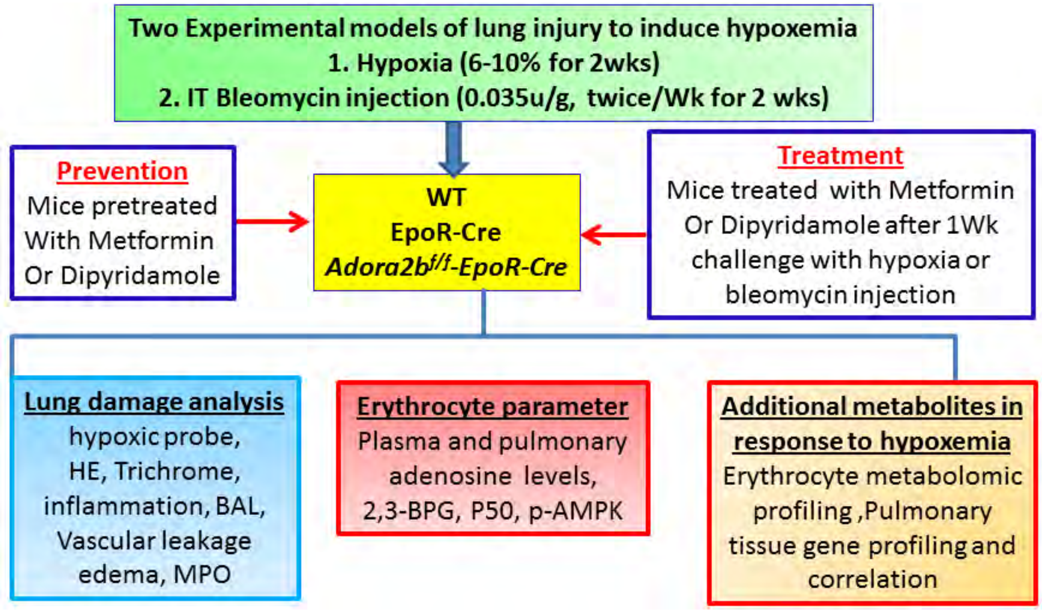

Fig.9. Preclinical studies: using two experimental models of lung injury to test prevention and treatment in mice with or without erythrocyte ADORA2B deficiency by metformin (Aim I) and dipyridamole (Aim II).

Anticipated Results. Because our early studies demonstrate that elevated circulating adenosine is beneficial for I.T. bleomycin-induced acute lung injury in mice[24] and our preliminary studies showed that adenosine signaling via erythrocyte ADORA2B promotes O_2 delivery by inducing 2,3-BPG production in a AMPK-dependent manner, we anticipate that 1) pretreatment of WT mice with metformin will induce 2,3-BPG and enhance O_2 release to lung tissues and thereby reduce or prevent hypoxia or bleomycin-induced lung injury and progression; 2) treatment of WT mice with metformin will induce 2,3-BPG and O_2 release and reduce pulmonary damage and slow disease progression; 3) metformin will restore adenosine-induced 2,3-BPG production and O_2 release and thereby rescue hypoxia or bleomycin-induced lung injury and progression in $AdoRA2B^{f/f}$-*EpoR-Cre* mice.

Potential Pitfalls and Alternatives: Besides metformin, AICAR is another potent AMPK agonist. Thus, in addition to metformin, we will also use AICAR as an alternative treatment in our proposed preclinical studies.

Q2. *What are other factors and metabolites involved in ADORA2B-mediated erythrocyte O_2 release to protect early hypoxia-induced lung damage and disease progression?*

Rationale. Hypoxia is an initial trigger to induce pulmonary damage and disease progression in COPD. Our innovative metabolomic screening combined with multidisciplinary approaches led us to successfully identify the detrimental effects of excess adenosine in SCD by promoting formation of deoxy-HbS, deoxy-HbS polymerization and sickling.[6] These findings les us to further discovered that AMPK is a key protein functioning downstream of ADORA2B underlying adenosine-induced 2,3-BPG production in normal erythrocytes. However, multiple factors and cellular systems are altered in response to hypoxia and may contribute to the pathogenesis of the disease. Erythrocytes are the most abundant circulating cells to deliver O_2 to local organs. Thus, they have been long speculated to be the most sensitive cells in response to hypoxia to protect tissue damage by promoting O_2 delivery. However, the specific factors and signaling pathways involved in hypoxia-mediated erythrocyte adaptive response to protect pulmonary damage and progression to COPD remains largely unknown.

Approach. Because erythrocytes have no nuclei, metabolomic profiling is the most powerful, accurate and unbiased high throughput methodology to assess the functional response of erythrocytes to hypoxia. Thus, in an effort to identify common metabolites and signaling networks altered in the erythrocytes from early stage to late stage hypoxia-induced pulmonary damage, we propose to conduct a non-biased high throughput metabolomic screening of erythrocytes using two independent lung injury models; induced by hypoxia and bleomycin at acute (24 hrs) and chronic stages (2 weeks) as described above. Next, to identify additional erythrocyte-specific molecules and metabolites induced by hypoxia or bleomycin we will also compare erythrocyte metabolomic profiles of *AdoRA2B$^{f/f}$-Epo-Cre* mice to the controls during disease development. In this way we hope to identify erythrocyte specific signaling molecules function downstream of ADORA2B. Moreover, we will conduct microarray to measure gene expression profiling in lung tissues of both controls and *AdoRA2B$^{f/f}$-Epo-Cre mice at acute and chronic stages*. Finally, we will correlate erythrocyte metabolomic profiles to pulmonary gene expression profiles in the mice.(**Fig. 9**)

Anticipated Results. We anticipate that unbiased erythrocyte metabolomic screening coupled with gene expression profiling in the lung tissues of two COPD models in both control and *AdoRA2B$^{f/f}$-EpoR-Cre mice* from early hypoxia to late stage will likely i) identify additional factors and metabolites underlying hypoxia-induced lung damage and progression; ii) reveal new pathogenic markers for early prediction of the disease progression; iii) provide novel therapeutic possibilities to treat and prevent disease progression.

Potential Pitfalls and Alternatives. We have successfully conducted metabolomic screening in SCD erythrocytes.[6,29] Thus, we are very familiar with all of the experiments we proposed and do not have any difficulty to perform the proposed studies.

Novelty and Overall Significance. The proposed studies are extremely innovative since the role of AMPK in the erythrocyte (particularly ADORA2B) in respiratory diseases has never been addressed. The proposed research is highly significant since it is likely to provide new insights regarding the importance of erythrocyte ADORA2B-mediated AMPK activation in physiological and pathological conditions associated with hypoxia and thereby facilitate and validate the use of metformin to enhance O_2 release to counteract hypoxia and prevent pulmonary damage and disease progression. Because it is impossible to analyze lung damage at different time points in humans, our proposed genetic and pharmacological studies will provide solid preclinical evidence of the therapeutic effects of metformin to promote O_2 release and reduce hypoxia, an initial event. We hope that our preclinical results pave the way for important clinical trials in humans utilizing the therapeutic approaches we have identified. The results obtained from translational human studies in Aim III are expected to provide support for our hypothesis that enhancing AMPK by metformin leads to increase O_2 release to reduce hypoxia in health humans.

Thus, the proposed preclinical studies combined with our exploratory studies in healthy humans in Aim III will set up a strong foundation for clinical trials to treat patients with respiratory diseases. Finally, nonbiased erythrocyte metabolomic screening and pulmonary gene expression profiling which is difficult to conduct in humans and will likely identify additional new modulators involved in hypoxia-induced lung injury, reveal early pathogenic biomarkers and provide innovative preventative and therapeutic possibilities for the disease at different stages.

Aim II. Conduct preclinical studies to determine the therapeutic effects of dipyridamole in hypoxia-induced lung diseases by increasing extracellular adenosine and erythrocyte 2,3-BPG and O_2 release.

Rationale. Dipyridamole is a FDA approved oral drug to inhibit ENTs leading to increased plasma adenosine. In particular, it has been used effectively to treat pulmonary hypertension patients without lowering systemic blood pressure. In a view of our finding that adenosine is increased in healthy humans under high altitude hypoxia and in normal mice under hypoxia and dipyridamole treatment induced 2,3-BPG production and O_2 release under normoxic condition in WT mice, we hypothesize that dipyridamole is likely a safe drug to increase circulating adenosine and enhance O_2 release by inducing 2,3-BPG to protect hypoxia-induced lung damage.

Approach. We propose to test the therapeutic effects of dipyridamole to prevent and treat lung damage in two mouse models as described in Aim I. Briefly, we will expose WT mice to different degree of hypoxia (6-10% O_2) or IT injection of bleomycin for different time points as above (**Fig. 9**). Specifically, i) *to assess if dipyridamole pretreatment can prevent hypoxia-induced lung damage (prevention),* WT mice will be pretreated with different dosages of dipyridamole[24] prior to exposure to hypoxia or IT injection of bleomycin; *ii) to test if dipyridamole can reduce pulmonary damage and slow disease progression (treatment),* we will start to treat WT mice after 24 hour 8% hypoxia challenge or 24 hour after bleomycin IT injection with different dosages of dipyridamole. At different time points, the erythrocyte parameters, adenosine levels and pulmonary functional, histological changes, lung inflammation and leakage will be measured as described in Aim I.[6] Moreover, the important downstream signaling cascade of adenosine-mediated AMPK activation will also monitored in erythrocytes by specific phospho-antibody[30] and ELISA. Finally, we will measure and compare newly identified erythrocyte metabolites by metabolomic screening and candidate genes in the lung identified by gene expression profiling in Aim I in WT mice treated with or without metformin with exposure of hypoxia or bleomycin IT injection.

Anticipated Results. Because our preliminary studies showed that dipyridamole induces plasma adenosine, erythrocyte 2,3-BPG production and O_2 release in WT mice under normoxia, we anticipate that dipyridamole treatment reduces hypoxia-induced lung damage by rapidly inducing circulating adenosine, erythrocyte 2,3-BPG and O_2 release to hypoxic lung tissues.

Potential Pitfalls and Alternatives. Dipyridamole is a safe FDA approved drug. Thus, we do not expect any obvious side effects in our proposed preclinical studies. However, if we do not observe obvious enhancement of circulating adenosine in two lung injury models, we will propose to use another FDA approved drug, deoxychoformycin (Pentostatin) which is a specific inhibitor for adenosine deaminase. It has been used for 30 years to successfully treat leukemia patients by quickly raising plasma adenosine levels and decreasing increased immune cells to normal levels in those patients without obvious side effect. We are extremely familiar with both dipyridamole and pentostatin therapies to raise circulating adenosine. With his expertise, we do not envision any difficulty to conduct proposed studies.

Novelty and Overall Significance. We believe that the results of the preclinical studies proposed under Aim II will provide solid evidence for the efficacy and safety of dipyridamole in the treatment of mice with lung damage. Dipyridamole has been safely and successfully used for many years to treat and prevent pulmonary hypertension in humans. Our current studies show that dipyridamole is well-tolerated by WT mice and treatments significantly increased plasma adenosine and erythrocyte 2,3-BPG and subsequent O_2 release under normoxia condition. The proposed preclinical studies are expected to provide important evidence for the therapeutic benefit of dipyridamole in respiratory diseases by improving O_2 delivery in mice during disease development that is impossible

analyzed in patients. Moreover, our proposed pilot human studies in health humans (Aim III) coupled with preclinical studies in lung damage models will help us to transition to clinical trials to treat patients with respiratory diseases rapidly. Finally, the animal studies will provide new insight into ENT-dependent regulation of circulating adenosine on erythrocyte function and the effects of inhibition of ENTs on gene expression, metabolomic profile and histological alteration in the lungs at different time points that is impossible to conduct in humans. These findings will reveal new insight for pathogenesis of the disease and open up innovative preventative and therapeutic possibilities for the disease at different stages.

Statistical Analysis for Aim I and Aim II. Experienced statisticians associated with the NIH-funded Center for Clinical and Translational Sciences in Houston will analyze the data. First, the levels of plasma adenosine, levels of erythrocyte 2,3-BPG, AMPK levels and pulmonary changes in two mouse groups treated or untreated (n=10-15 for each group) will be analyzed by Student's *t* tests (paired or unpaired as appropriate) applied in two-group analysis. Differences between the means of multiple groups at different time points or different dosages of drugs will be compared by one-way analysis of variance, followed by a Turkey's multiple comparisons test. P value of less than 0.05 will be considered significant. The relationship between two variables X and Y will be analyzed by Pearson product-moment correlation coefficient method. $P<0.05$ (two-sided) will be considered statistically significant. The linear correlation (dependence) is described as R square.

Aim III. To use metformin and dipyridamole to elevate oxygen delivery in healthy humans experiencing hypoxia to examine the consequences on physical and cognitive performance, and to choose the most effective approach to manipulating oxygen delivery for future studies on COPD patients.

Rationale. Based on our recent preclinical and human field studies described above, improving oxygen delivery to hypoxic tissues by manipulating the newly discovered ENT-Adenosine-AMPK signaling network has the potential to completely change the clinical approach to managing hypoxia. In Aim III we will do the first studies in humans to examine the effectiveness of manipulating the ENT-Adenosine-AMPK signaling network to improve oxygen delivery in humans. As a first step on the path to eventual clinical trials in COPD and other patients with hypoxia-associated respiratory disease, we will study the exercise and cognitive function effects in healthy humans acutely exposed to terrestrial hypoxia while taking placebo, metformin or dipyridamole. Through different mechanisms we propose that metformin and dipyridamole will have the ultimate effect to improve oxygen delivery. Here we briefly review the background on human studies of metformin and dipyridamole that establish the feasibility and rationale for trying these drugs to elevate oxygen delivery in healthy, hypoxic humans.

Why Studying Healthy Subjects Is A Necessary First Step To Translate ENT-Adenosine-AMPK Signaling In Patients with Respiratory Disease? To date we know that the ENT-Adenosine-AMPK signaling seems remarkably similar in hypoxic mice and humans. We know that in healthy humans, the end-product of activation of this pathway in the form of elevated 2,3-BPG and P50 levels, is strongly related to overcoming hypoxia. And we know from mouse studies that metformin and dipyridamole, acting through different mechanisms, boost the activity of the ENT-Adenosine-AMPK signaling pathway resulting in greater O_2 unloading at the tissue. But we do not know the effects of these two drugs on healthy but hypoxic humans. The major advantage of studying these drugs first in healthy but hypoxic humans is that comorbid conditions can be ruled out, and heavy exercise that stresses the oxygen delivery system can be performed to reveal how a healthy biological system will react to elevations in O_2 unloading. Additionally, we do not know yet the most sensitive whole body physiological measurements to changing degrees of oxygen delivery. These questions will all be addressed in our proposed human studies, and will lay the foundation for near-term studies in patients ill with mild to moderate COPD.

Metformin to Improve Oxygen Delivery. Metformin is an FDA approved drug used to treat diabetic patients that decreases hyperglycemia primarily by suppressing glucose production by the liver. Although the molecular basis of metformin is not fully understood, it is well known to activate PKA and AMPK.[30-34] Based on our initial observation that PKA and AMPK are essential regulators of 2,3-BPG induction and thereby promote oxygen release from erythrocytes, we believe that metformin will improve oxygen delivery and physical work capacity in

those with compromised ability to transport oxygen. Before translational work can be performed in humans with respiratory disease, it is necessary to identify physiological tests that are sensitive to metformin-induced effects in healthy, but hypoxemic individuals. Because repiratory disease is characterized by systemic hypoxia, we will study healthy individuals exposed to hypoxia at high altitude as a model of compromised oxygen delivery.

The effect of metformin on oxygen transport in healthy, non-diabetic individuals has received little attention.[35,36] Peak workload achieved during exhaustive cycling tests was not different from placebo, but oxygen consumption was slightly less on metformin.[35] These results suggest that metformin improves mechanical efficiency (more power output for a given amount of oxygen consumption) during high-intensity exercise. We find this effect intriguing because similar improvements in mechanical and mitochondrial efficiency have been reported to explain increases in exercise performance subjects acclimatize to hypoxia at high altitude.[37-39] Additionally, metformin therapy has recently been shown to increase cognitive function in diabetic patients.[29,40] These results support the neuro-protective effects of metformin reported in animals and cell cultures.[41,42] Based on similarities between metformin's mechanism of action and the molecular processes of acclimatization, we believe metformin will improve oxygen transport in those challenged by hypoxia at high altitude. In this phase of the study, we will determine the most sensitive measurements to document metformin-induced effects for future translational work in a patient population.

Dipyridamole to Improve Oxygen Delivery: In contrast to metformin's frequent use for treatment of diabetes, dipyridamole is used most frequently in humans for vasodilation in cardiac function studies. As far as we are aware it has not been evaluated for altering exercise performance in any setting, nor in any way at high altitudes or in hypoxia. Dipyridamole is well-tolerated with an excellent safety profile.[43]

Approach. We will follow a standard experimental model (double-blind, placebo controlled, matched cohort design) to test the hypotheses metformin and/or dipyridamole will improve indices of arterial and tissue oxygen delivery at high altitude. We expect that these improvements will increase physical and cognitive performance and prevent mountain sickness during a simulated, rapid deployment to high altitude (~10,000 to 13,000 feet).

Subject Recruitment. Following approval from local IRBs and DoD Human Research Protection Office, 100 individuals will be recruited from student populations near sea level in central Michigan (Alma College, Central Michigan University, Michigan State University). The major inclusion criteria will be: healthy men and women 18-40 years old who can meet physical fitness requirements for the US Army (see below). The major exclusion criteria will be: those with anemia; those with known disease; those with a history of significant head injuries or migraines; those who are unable to achieve the minimum physical fitness standards; those taking any medications that interfere with oxygen delivery and transport (including sedatives, sleeping aids, tranquilizers, diuretics, alpha and beta blockers, and any medication that depresses ventilation), and those with known allergies to sulfonamide-based drugs. All potential subjects will give written informed consent prior to the competitive selection procedures listed below.

Physical Screening. Eligible volunteers consenting to the protocol will undergo physical examinations, including blood draws for standard blood chemistry, and perform the Army Physical Fitness Test (APFT) to verify health and fitness standards. Specifically, all participants must be able to score a minimum of 60 points based on each of the three age adjusted APFT criteria for push ups, sit-ups, and 2-mile run.

Sea Level Testing. The top 70 scoring individuals (~35 males, ~35 females) on the APFT will undergo further laboratory testing to familiarize themselves with the experimental procedures at high altitude. Subjects resting arterial saturation (pulse oximetry), heart rate, symptoms of AMS (Lake Louise Questionnaire), and cognitive performance (DANA) will be assessed a minimum of two times. Subjects' steady-state metabolic responses at three submaximal work rates (5 min at 50, 75, and 100 watts for women or 50, 100, and 150 watt for men) and peak oxygen consumption (25 watt/min ramp protocol) will be assessed during cycle ergometry. During the test, metabolic rate (indirect, open-circuit calorimetry) and regional oximetry (near infrared spectroscopy) will be used

to determine oxygen consumption, tissue (cerebral and muscle) oxygenation, and metabolic efficiency.

Group Assignments. Sixty subjects will be matched according to sex, height, weight, and oxygen consumption to form three groups of 20 subjects who have similar sea level characteristics. These groups will be randomly assigned to the three treatment groups: placebo, metformin, and dipyridamole.

Drug Administration. Subjects will begin taking oral medications 48 hours prior to their scheduled departure from sea level and continue treatment through their stay at high altitude. All compounds will be prepared and coded by a clinically licensed pharmacy. Investigators and subjects will be blinded to the identity of the compounds until after the study is completed. Those in the metformin group will take 500 mg once a day for 48 hours, then 500 mg twice daily while at altitude. This uptitration schedule is commonly used to minimize potential gastrointestinal discomfort.[35,44] Those in the dipyridamole group will take 200 mg twice daily while at altitude. Those in the placebo group will take a non-physiologically active substance (cellulose) packaged in identical capsules.

High-Altitude Testing. Subjects will be transported to Denver, Colorado in groups of 8-12 by commercial airlines and then immediately driven to Breckenridge, Colorado by charter buses. The total travel time from sea level to high altitude will be ~6 hours. Subjects will follow a strict regimen of tasks designed to simulate military-relevant physical activity at high altitude (10,000 to 13,000 feet) over two days and two nights before returning to Michigan on the third day, as described below.

> **Day 1.** Upon arrival at high altitude (~12 pm MST), subjects' resting arterial saturation, heart rate, and AMS symptoms will be assessed. Subjects will then perform a 3.1-mile uphill hike as fast as possible while being timed. The course follows a rugged hiking/jeep trail that begins in a wooded area at 10,627 feet and ends on a ridge above tree line at 12,595 feet. Fit subjects, free of AMS, can finish the course in ~60 minutes. That evening (~ 8pm MST), in addition to measurements of resting arterial saturation, heart rate, and AMS symptoms, subjects' cognitive performance will be assessed.
>
> **Day 2.** In the morning, following AMS symptoms scoring, subjects will have a radial artery catheter placed in their non-dominant wrist. One ml of blood will be sampled to measure resting arterial blood gases, pH, P50, lactate, and glucose. Subjects will then repeat the laboratory exercise tests to assess oxygen consumption, tissue (cerebral and muscle) oxygenation, and metabolic efficiency, as described above. Arterial blood samples will be taken at the end of each steady-state work rate and at peak oxygen consumption. That evening, subjects will repeat the arterial saturation, heart rate, AMS symptoms, and cognitive function assessments.
>
> **Day 3.** Following morning measurements of arterial saturation, heart rate, and AMS symptoms, subjects will be transported back to sea level.

Data Analysis. The primary outcome measures of steady-state oxygen consumption, metabolic efficiency, and arterial and tissue (cerebral and muscle) oxygenation obtained during cycle ergometry, along with uphill running and cognitive performance, at high altitude will be analyzed using one-way ANOVA with planned comparisons to determine the effects of the drugs relative to placebo. This method of analysis considers variance across all treatments, but controls for type I error ($\alpha = 0.05$). Chi square analysis will be used to evaluate the incidence of AMS. An a priori power analysis based on the expected incidence of AMS in the placebo (50%) and experimental (0%) treatments, revealed that 14 subjects per group would be necessary to detect a meaningful positive outcome. Similarly, using estimates of peak oxygen consumption based on 2-mile run performance at high altitude from one of our previous studies (44.5 ± 6.2 ml/kg/min), 10 subjects per group would be necessary to detect a 4% improvement if the drugs have a moderate to large effect (Cohen's d = 0.70, $\alpha = 0.05$). Assigning 20 subjects to each group (N=60) is thus expected to maintain statistical power at 80%.

Anticipated Results. We anticipate that compared to the placebo group, both medications will cause elevation of 2,3-BPG levels, and that the higher the 2,3-BPG levels will be correlated with greater submaximal exercise performance, fewer and less severe symptoms of altitude illness, and better oxygen transport reflecting better tissue O2 delivery at rest and during maximal exercise.

Potential Risks and Solutions. Specific technical risks and challenges to the successful and timely execution of this study include: multi-site Institutional Review Board (IRB) approvals, subject recruitment and retention, and inducing sufficient severity of altitude illness. The extent of many of these risks is similar to that typically experienced with any research study. The 34+ years of experience in this field of Dr. Roach, the Partnering Principal Investigator,[45-50] and the experience of the Co-Investigators,[45-50] significantly reduces not only common research risks, but also the specialized risks associated with a human hypoxia field study. The details for risk mitigation are detailed below.

Multi-Site IRB. The requirement for research protocol approval by three unrelated agencies (University of Colorado Denver, Alma College, and DOD's Human Research Protections Office) puts at risk the timely execution of this study. Our timeline has built in six months for the three IRB processes to ensure ample time for rigorous review. The Altitude Research Center staff has extensive experience in altitude sickness research ethics, and has secured human subjects approval for several high-altitude protocols of this nature; this will help facilitate timely IRB approval. We are already beginning the IRB process for this study; this action, plus the six months in the funded portion of the study, allows more than 1 year for IRB approval. Other project tasks preceding subject recruitment (see Statement of Work) will be completed in parallel to ensure the protocol can begin immediately upon final IRB approval. This will reduce the risk of a longer than usual IRB process that could cause delay in completion of the study.

AMS Severity. The altitude we propose for AMS induction is well proven. All subjects will experience a nearly identical altitude exposure from sea level in Michigan to ~10,000 feet in Colorado within ~6hrs. Incidence of AMS at this altitude in a group of conference attendees, more sedentary and experiencing more gradual transport than our subjects, was 28%.[48] In our recent DoD-funded studies, AMS incidence was about 40%, probably due to slightly higher altitude than the conference attendees experienced, and the high level of physical exertion we expose our subjects to once they are at high altitude. Our sample size and power analyses assure enough AMS-positive cases in each of the three groups (see Approach, above, for details).

Subject Recruitment and Retention. Our subject recruitment and retention success from our recent altitude chamber studies, and from numerous field studies originating at low altitude but including a remote field study alleviate any concerns regarding recruitment setbacks. In particular, our chamber study protocol including three, 10-hour chamber exposures separated by a minimum of 4 weeks and extensive physiological testing, and our extensive recent experience on DoD-funded field studies[45-50] demonstrates our ability to retain subjects in a markedly more lengthy protocol than those we propose here. Based on this experience, the risk related to subject recruitment and retention is extremely low.

Summary. We have presented experiments to investigate the mechanisms and efficacy of treatment and protective roles of elevating tissue O_2 delivery in hypoxia-induced lung injury in mice in Aims I and II. And in Aim III we translate our previous findings of elevated 2,3-BPG in hypoxic humans associated with improved tolerance to hypoxia in an experimental paradigm that, if successful, can be easily translated to patients with hypoxia secondary to respiratory diseases.

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List of Abbreviations, Acronyms and Symbols

COPD: chronic obstructive pulmonary disease; **adenosine**: Adenosine; **2.3-BPG:** 2,3-diphosphoglycerate; **O_2**: oxygen**; AMPK**: AMP activated protein kinase; **ENTs**: equilibritive nucleoside transporters; **ADORA2B**: adenosine A2B receptor; **FDA**, Food and Drug Administration

FACILITIES, EXISTING EQUIPMENT AND OTHER RESOURCES

The University of Texas Health Science Center at Houston

Laboratory
I have been assigned approximately 1200 square feet of laboratory space that include 8 large laboratory workbenches and 8 laboratory desk areas. In addition, I have access to specially equipped laboratories of cell culture work and mouse manipulation. All of these rooms are located near each other along the same corridor.

Clinical
None

Animal
Animal facilities are available through the University Center for Laboratory Animal Medicine and Care. We have an approved animal protocol to perform all the experiments proposed here. My laboratory has *EpoR-Cre* and novel *Adora2b*$^{loxp/loxp}$/*Epo-Cre* mice. These genetic tools will allow us to examine the mechanisms used by metformin and dipyridamole for raising tissue O_2 delivery and thus preventing lung damage.

Computer
I have a desktop and a notebook computer for my personal use. Each computer is equipped with appropriate software for internet and email access, word processing and graphics applications. Additional computers are associated with specific laboratory instruments. All personnel associated with this project have a notebook computer for their personal use.

Office
I have a private office of approximately 200 square feet. The office is located near my assigned laboratory space. Secretarial and clerical assistance are available through the department's central office.

Other
The UT Medical School supply mall is located in the basement of the same building in which my labs are. This mall is a centralized facility where consumables, chemicals and molecular biology reagents from all major vendors are available for immediate purchase. This facility greatly enhances the pace at which research can be conducted.

The scientific environment of UT-Houston Medical School is excellent and is enhanced by its presence in the Texas Medical Center that contains other outstanding research institutes including Baylor College of Medicine and MD Anderson Cancer Center. There is a growing community in The Texas Medical Center interested in various aspects of pulmonary related disease. In addition, there is a large collection of scientist interesting in erythrocyte physiology and blood disorders. These features have and will continue to enrich the research conducted in my laboratory.

Significant resources and capabilities are provided by our CTSA (locally named CCTS, or Center for Clinical and Translational Sciences). *a) Statistical support service-*The translational studies proposed here in Aim III will benefit from the professional statistical assistance associated with our CCTS-BERD. b) *Cost effective research services-*Our CCTS provides a number of cost effective services in various analytical areas relevant to the biomarker research proposed here. Of particular importance are the metabolomic, proteomic, microarray, genomic and qPCR cores. The use of these biomarker cores provides cost saving for our institutional investigators.

Major Equipment
1. HPLC to measure adenosine in our department.
2. Hemoxy Analyzer to measure deoxyl-and oxy-hemoglobin and oxygen equilibrium curve.

3. Hypoxic chamber.
4. Ten color-flow cytometry through core laboratories at the medical school.
5. Animal core facility provides us to complete blood count, kidney function and liver function by blood measurement.
6. A microscopy suite for mouse tissue histology that includes equipment for tissue fixation, embedding, sectioning and fluorescent and light microscopy with digital image processing.
7. Dissecting microscopes for microsurgical procedures.
8. Anesthesia equipment for mice.
9. Quantitative RT-PCR are available through the services of a core laboratory here at the medical school.
10. CODA High-Throughput NIBP system-8 Channels to efficiently measure blood pressure in the mice.
11. Oxystat from CODA to measure Percentage of saturated hemoglobin in vivo.
12. A complete set of instrument to measure right ventricle systolic pressure from AD instrument.
13. A complete set of instrument to measure systolic blood pressure from AD instrument.
14. Metabolic cages to collect 24 hour urine from mice.
15. Microplate Reader
16. Cell culture hood and incubator.
17. Different speed centrifuges.
18. Multiple Western blot apparatus.
19. Ultrasonographic device to measure blood perfusion in mice.
20. Spectrophotometer

ARC Facilities and Equipment

The Altitude Research Center (ARC) is situated on the Anschutz Medical Campus (AMC) of the University of Colorado Denver (UCD), in Aurora, Colorado. The ARC laboratory facility, a 5,000 square foot center, is dedicated to integrative physiology research in humans.

The ARC is a research facility equipped with specialized altitude related research equipment. The ARC is configured of eight offices, a conference room, a break room, an examination room, and two laboratory spaces. One of the two lab spaces (chamber room) includes a hypobaric chamber facility capable of hosting up to eight research subjects and investigators at altitudes up to 120,000 feet for 12-24 hours. The second laboratory space is reserved for experiments performed at Denver's altitude. This laboratory space is capable of a wide range of research testing, including but not limited to muscle strength and endurance experiments, as well as biomechanical analysis and energy expenditure.
The Chamber Room, with separate Vacuum Pump Room, connects to the main laboratory. This room houses the 12ft x 28ft modern hypobaric chamber capable of hosting 2-8 research subjects and investigators at altitudes up to 25,000 feet for 12-24 hours.

Core Equipment includes:

1. Oxymon MkIII near infrared spectrometer for tissue oxygenation measurements.
2. Oxymon MkII near infrared spectrometer for tissue oxygenation measurements.
3. Spencer ST3 transcranial Doppler for measurement of cerebral blood flow velocity.
4. DWL Transcranial Doppler for measurement of cerebral blood flow velocity.
5. Sonosite Micromaxx diagnostic ultrasound for monitoring vascular blood flow and cardiac output.
6. Nexfin HD finger plethysmograph for beat-by-beat blood pressure and cardiac output measurements.
7. Colin 7000 tonometer for beat-by-beat blood pressure monitoring (x2)
8. Respiract respiratory gas mixer for controlling end-tidal concentrations of oxygen and carbon dioxide.
9. Ametek oxygen (S-3a/II) and carbon dioxide (CD-3A) analyzers for metabolic measurements (x2).
10. Oxigraf O2cap oxygen and carbon dioxide analyzers for metabolic measurements (x2).
11. Powerlab 16SP and 16/30 data acquisition systems.
12. Radiometer OSM-3 hemoximeter for hemoglobin and hematocrit measurements (x2).
13. Laboratory Instruments blood gas analyzer.
14. Velotron Elite cycle ergometer for time trial exercise testing.

Minor equipment in the main ARC laboratory includes: Nellcor N-595 (measures oxyhemoglobin saturation in the peripheral circulation (2 each)), Criticare 503 (measures oxyhemoglobin saturation in the peripheral circulation (2 each)), Universal Ventilation Meter (measures ventilation via spirometry), O_2Cap Oxygen Analyzer (measures oxygen and carbon dioxide concentrations (2 each)), Powerlab 16/30P and Power lab 16SP (both able to integrate up to 16 analog inputs into a single, time-aligned data file and allowing for real-time and offline manipulation of this data), Ametek O_2 and CO_2 analyzers (measures oxygen and carbon dioxide concentrations (2 each)), Vacuumed Metabolic Measurement System (measures ventilation, respiratory gases, and oxygen consumption (2 each)), SECA portable scale (weight measurement of research participants during study), as well as a Samaritan SED defibrillator pad (for basic life support). In addition we will set up a Sorvall RT 6000 Refrigerated Centrifuge (allows for the separation of 15-50 mL tubes at speeds of up to 6,000 revolutions per minute), the Jouan BR 3.11 Centrifuge (separates 5-50mL tubes), and the LW Scientific Microhematocrit Centrifuge (spins down twenty-four 75mL capillary tubes).

Offices**:** Offices are located to the sides of the main hallway of the ARC, and range from 233 sq feet to 115 sq feet. All offices are equipped with an individual telephone line, internet access through the Universities hard-wired high-speed access. In addition a wireless router allows for Internet access through the Universities wireless network. All offices contain computers, desks, chairs and filing cabinets for their occupants. The conference room is 280 sq. ft and is equipped with a dry-erase whiteboard, large projection screen and LCD projector.

UTHealth
The University of Texas
Health Science Center at Houston

Medical School
Department of Biochemistry & Molecular Biology

Rodney E. Kellems, PHD
Professor and Chair

October 10, 2014

Department of Defense-CDMRP

Re: Yang Xia, M.D., Ph.D.- DOD Application

To Whom It May Concern:

The purpose of this letter is to express my enthusiastic support for Dr. Yang Xia's application for a DOD Award. Dr. Yang Xia is currently appointed as Professor in the Department of Biochemistry and Molecular Biology at The University of Texas Health Science Center at Houston-Medical School, a position she is expected to hold throughout the period covered by the DOD award that she is seeking. Our continued commitment to Dr. Xia as an independent faculty member is not contingent on receipt of this award.

To assist Dr. Xia in her research, we have provided her with office and laboratory space. In addition, she has full access to common equipment within the department, the animal facility housed within the Medical School, and as a faculty member she is entitled to the research administrative support provided by the central office in our department. In addition to her ongoing association with other faculty members in our department, she will benefit from interactions with investigators throughout the Texas Medical Center, with whom she has already established research collaborations. I feel Dr. Xia's research program is highly significant and that our research environment will promote the funding and other successes that will be necessary for her research to continue.

Please accept this letter as confirmation of the laboratory space, equipment, and other resources available for this proposed project . I appreciate your thoughtful consideration of her request for DOD funding

Sincerely,

Rodney Kellems, PhD
Professor and Chairman
Department of Biochemistry and Molecular Biology

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September 18, 2014

To whom it may concern,

I am writing in support of Dr. Roach's application "Innovative Metabolomic Profiling Reveals Novel Approaches to Promote Oxygen Delivery in Respiratory Disease" and can certify that the required laboratory space, equipment and other resources for this project are available to the PI.

Please do not hesitate to contact me if you need any further information

Sincerely,

Richard D. Zane, MD
Chair

Medical School
Department of Biochemistry & Molecular Biology

October 10, 2014,

Yang Xia, M.D., Ph.D.
Professor
Department of Biochemistry and Molecular Biology
The University of Texas-Houston Medical School
6431 Fannin
Houston, TX 770303

Dear Yang,

I have enjoyed collaborating with you for the last ten years. I am writing to express my strong support for your current proposal entitled "***Innovative metabolomic profiling reveals novel approaches to promote oxygen delivery in respiratory disease***". My laboratory will be able to provide you with expertise assessing therapeutic effects of metformin and dipyridamole-mediated oxygen delivery in lung injury by running your erythrocyte specific knockout mice through multiple models of lung injury as you proposed in the grant. As you know my laboratory has substantial expertise and the appropriate infrastructure to conduct these experiments. I am extremely excited about therapeutic possibilities of using these two FDA approved drugs to treat respiratory disease. By working closely with you to assess oxygen delivery in these models, we anticipate obtaining important information into the protective roles of ADORA2B and ENTs in acute lung injury and disease progression.

I look forward to a continued productive relationship.

Best Regards,

Michael R. Blackburn, Ph.D.
Professor

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Oct 15, 2014

To: Dr. Robert Roach

From: Mr. Rod Alne

Re: Letter of Support for *"Innovative metabolomic profiling reveals novel approaches to promote oxygen delivery in respiratory disease" by Dr. Robert Roach, Partnering PI.*

I am pleased to support the proposal to investigate the testing of ways to improve tissue oxygen delivery to treat and prevent lung disease in animal models, and to improve oxygen delivery in hypoxic but otherwise healthy humans. I can attest to the importance for Special Operations Forces to have some new ideas in this area, especially an approach that would improve both physical and cognitive performance while protecting from high-altitude illnesses. That would present a major breakthrough!

I retired from the Air Force as a Chief Master Sergeant with over 27 years in Pararescue. The The Peak was started in 2005 to address shortfalls in military training that I recognized while deployed to Afghanistan in support of Operation Enduring Freedom.

The Peak Inc. is a Service-Disabled Veteran Owned, certified by the Veteran Administration, and operated business developed to address Special Operating Forces (SOF) concerns and increase their effectiveness while operating in austere environments and the extremes of combat. The Peak provides world class, high altitude training to ensure maximum performance during extended periods of operation in austere environments.

I look forward in working with you and your team if this project gets funded. In particular, I would bring my real world experience to the consulting with you on the design of the field experiments. Though there are real world limits as to what can be simulated in civilians in the field, you and your team have demonstrated a strong ability to conduct state of the art and militarily-relevant human research. You are the best at what you do. Working together again would be a pleasure.

I look forward to contributing to this exciting project!

Sincerely,

INSTITUTE FOR ALTITUDE MEDICINE
AT TELLURIDE

October 15, 2014

To: Dr. Robert Roach

From: Dr. Peter Hackett

Re: Letter of Support for *"Innovative metabolomic profiling reveals novel approaches to promote oxygen delivery in respiratory disease" by Dr. Robert Roach, Partnering PI.*

I am very pleased to support the proposal for testing ways of improving tissue oxygen delivery to treat and prevent lung disease in animal models, and to improve oxygen delivery in hypoxic but otherwise healthy humans. If your approach using metformin and dipyridamole to acutely increase 2,3-BPG and thus achieve better tissue oxygen delivery works, it would be a major breakthrough, with applications to patients and healthy soldiers!

I look forward to working with you and your team if this project gets funded. In particular, I would bring my real world experience as a specialist in mountain medicine to the process of designing the field experiments. You and your team have demonstrated a strong ability to conduct state of the art and militarily relevant human research. Working together again would be a pleasure.

I look forward to contributing to this exciting project!

Sincerely,

Peter

Peter Hackett MD
Director, Institute for Altitude Medicine, Telluride
Clinical Professor of Emergency Medicine, University of Colorado Denver,
Altitude Research Center, University of Colorado Denver

970-728-3848 · P.O. BOX 1229 · TELLURIDE, CO 81435 · INFO@ALTITUDEMEDICINE.ORG

Intellectual property

No background intellectual property will be used.

Data and Research Resources Sharing Plan

We will deposit all data in appropriate public repositories, and share data with all interested investigators.

Attachment 3. Technical Abstract

Background: This proposed project in RESPIRATORY HEALTH tests two FDA-approved drugs for their effectiveness in treating hypoxia in respiratory disease by elevating tissue oxygen (O_2) delivery. Hypoxia is a major contributor to and consequence of many respiratory and cardiovascular diseases. For example, hypoxia is a major component of chronic obstructive pulmonary disease (COPD), a clinically devastating disease of increasing prevalence, occurring more in military veterans than in the general population.[1-3] And many COPD patients, even those with mild disease, regularly suffer additional hypoxia during sleep.[4] Healthy troops can also be exposed to hypoxia through deployment to high altitudes (e.g. to mountainous regions of Afghanistan or in CONUS at Fort Carson, Colorado). Thus, for DoD personnel, veterans, and patients with COPD, tissue O_2 delivery is limited. Limitations in tissue O_2 delivery decrease overall well-being and cognitive function, and directly causes poor physical performance (even walking ability is limited).[5]

Rationale and Hypothesis: Our work described above leads to the compelling hypothesis that increasing extracellular adenosine by enhancing AMPK activation by metformin or by inhibition of ENTs by dipyridamole resulting in elevated levels of 2,3-BPG are promising new approaches to improving tissue O_2 delivery in patients with respiratory disease and in hypoxic healthy subjects. In the proposed studies we extend our current discoveries by conducting preclinical studies to determine the therapeutic effects of metformin and dipyridamole in two independent experimental models of lung injury (Aim I and II) and conduct mechanistic studies by identifying additional factors and signaling pathways involved in erythrocyte ADORA2B-mediated O_2 release in response to hypoxia using our newly developed erythrocyte mouse line (Aim I). We also propose to test if metformin and dipyridamole improve tissue O_2 delivery and counteract hypoxia in hypoxic but otherwise healthy humans in Aim III.

Objective: The major goal of our proposed research is to conduct both preclinical animal and human studies to translate our new discovery of ways to elevate tissue O_2 release to treat hypoxia.

Specific Aims: To accomplish our goal, we propose three specific aims: In Aims 1 and 2 we will determine if metformin and/or dipyridamole can reduce hypoxia-induced lung damage, and identify the molecular basis for of these drug's actions. And in Aim III, we will use metformin and dipyridamole to elevate oxygen delivery in healthy humans experiencing hypoxia to examine the consequences on physical and cognitive performance, and to choose the most effective approach to manipulating oxygen delivery for future studies on COPD patients.

Research Strategy: In Aim I & Aim II, we will test the therapeutic benefit of metformin & dipyridamole in respiratory diseases by improving O_2 delivery from erythrocytes at different stages of the disease. Additionally, we will use our newly developed novel mouse lines coupled with metabolomic and gene expression profiling to identify additional new modulators underlying hypoxia-mediated adenosine response during the progression of disease. For Aim III, since both metformin & dipyridamole are FDA approved drugs, we propose to conduct pilot human studies to test its beneficial role in humans in under hypoxia. In summary, the preclinical studies and human pilot studies will pave the way for future clinical trials to treat and prevent COPD.

Impact: Our research builds on novel translational findings that have the potential to offer a completely new approach to managing hypoxia, and even treating and preventing some hypoxia-related respiratory diseases. The impact of proposed research is highly significant since it is likely to provoke a paradigm shift in our understanding of the molecular mechanisms of improving O_2 delivery in any condition involving systemic hypoxia. In particular, if our preclinical studies and pilot human studies show the beneficial role of metformin and dipyridamole in hypoxia, transition to future clinical trials in COPD or other hypoxic respiratory diseases should be rapid. We are submitting this proposal under the **Partnering PI** option. Our unfunded yet successful collaboration thus far has revealed important new insights into how humans adjust to low O_2. We are individually and even more so as a team very productive researchers with both extensive NIH funding (Dr. Xia) and DoD funding (Dr. Roach), and with strong, high impact publications in this topic area[6,12-17,19,51-54]. We propose that this research will lead to even better and more extensive new findings on O_2 transport to benefit anyone experiencing hypoxia, from the veteran with COPD to the soldier deployed to high altitude.

Attachment 4. Lay Abstract

The topic area of this proposal is Respiratory Health. The central critical problem we are addressing is low oxygen, also known as hypoxia, in the body. Humans can experience hypoxia for a variety of reasons, including as part of many lung and heart diseases. Also, soldiers who live and work at high altitudes, like in Afghanistan, can experience hypoxia. You can treat hypoxia by breathing in supplemental oxygen, or by descending to lower altitudes. But there is one more possibility, and that is the focus of this proposal. Oxygen is transported from the air through the lungs to the blood, and it then combines with hemoglobin to be transported throughout the body. The chemistry of how oxygen and hemoglobin combine can be manipulated to change how tightly hemoglobin picks up oxygen in the lungs and how effectively it lets go of oxygen when it is ready to delivery the oxygen to a tissue, like your heart muscle or leg muscle to provide energy those tissues need for work.

We have discovered some of the details of the mechanisms of how hemoglobin lets go of oxygen at the tissue. We have also shown that when healthy people adjust to hypoxia these mechanisms are very active. The main idea of this project is to make those mechanisms of oxygen delivery more active, more efficient and thus to boost the overall quantity of oxygen delivered in animals and in humans. We will try to make those mechanisms more active by using two drugs, metformin and dipyridamole. These drugs are already approved for human use by the FDA, so if they are effective we can immediately start studies in patients. In the animal studies we will see if our ideas can prevent or treat some common types of lung disease that are associated with hypoxia. And in humans, we'll see if we can improve how the body exercises and the brain thinks in high-altitude hypoxia.

If these trials are successful we will immediately try these drugs in patients with mild then with more severe lung disease. The potential is that a patient who now has to use oxygen to go food shopping could be free from supplemental oxygen use by using one of these medications. Or that the soldier who cannot exercise at the same level as the high-altitude adjusted enemy, will experience much better exercise performance when deployed to high altitude.

The impact of this proposal if successful is to transform the management of hypoxia in humans, both in the DoD and in civilian populations, in patients and in healthy people who are living and working at high altitude.

STATEMENT OF WORK

Site 1- Dr. Xia
Biochemistry and Molecular Biology Department
University of Texas Medical School at Houston

Site 2 – Dr. Roach
Altitude Research Center
University of Colorado School of Medicine

Overall Project Management	**Timeline** months	**Site 1**	**Site 2**
Bi-weekly phone and monthly Skype project coordination meetings between Xia and Roach	1-36	Xia	Roach
Strategic planning for joint publications, student opportunities in both laboratories and coordination of postdoc efforts in support of the project.	1-36	Xia	Roach
Strategic planning for all regulatory compliance issues for animal and human studies, share expertise between laboratories and Universities.	1-36	Xia	Roach
Specific Aim 1: Conduct preclinical studies to determine if metformin can reduce hypoxia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling in hypoxia-induced lung damage and disease progression.			
Major Task 1: Xia Study Preparation			
Subtask 1.1: Obtain AWC animal protocol approval at local and DOD level (months 1-3 before start of funding). *Outcome: animal protocol approval from Univ of Texas-Medical School at Houston and DOD.*	1-3		
Subtask 1.2: Subtask 2: Set up a large of mating pairs to produce enough genetic deficient mice for Aim I. (3 months)	1-3		
Subtask 1.3: Purchase and ship wild type mice from Jackson's laboratory.	1-3		
Major Task 2: Conduct preclinical studies to determine if metformin can reduce hypoxemia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling in hypoxemia-induced lung damage and disease progression.		Xia	
Subtask 2.1: To assess the preventive and treatment effects of metformin on hypoxia-induced lung damage model.	4-8		
Major Task 3: Conduct preclinical studies to determine if metformin can reduce hypoxemia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling in hypoxemia-induced lung damage and disease progression.		Xia	
Subtask 3.1: To assess the preventive and treatment effects of metformin on bleomycin-induced lung damage model.	8-12		
Milestone: *Provide preclinical evidence whether metformin is a safe and effective drug to prevent and treat lung tissue damage and progression in WT mice by inducing 2,3-BPG production and O2 release. Discuss results.*		Xia	Roach
Major Task 4: Conduct preclinical studies to determine if metformin can reduce hypoxemia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling		Xia	

in hypoxemia-induced lung damage and disease progression.			
Subtask 4.1: To assess if metformin rescue hypoxia-induced lung damage model in *Adora2b$^{f/f}$-EpoR-Cre* mice by increasing AMPK activity, 2,3-BPG levels and improving O2 delivery	12-16		
Subtask 4.2: To assess if metformin rescue bleomycin-induced lung damage model in *Adora2b$^{f/f}$-EpoR-Cre* mice by increasing AMPK activity, 2,3-BPG levels and improving O2 delivery	12-16		
Milestone: *Provide genetic evidence whether metformin rescue lung tissue damage and progression in Adora2bf/f-EpoR-Cre by inducing 2,3-BPG production and O2 release. Discuss results.*		Xia	Roach
Major Task 5: Conduct preclinical studies to determine if metformin can reduce hypoxemia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling in hypoxemia-induced lung damage and disease progression.		Xia	Roach
Subtask 5.1: Compare metabolomics profiling in erythrocytes isolated from WT, EpoR-Cre and *Adora2b$^{f/f}$-EpoR-Cre* under normoxia *or hypoxia or with injection of vehicle or bleomycin.*	16-28	Xia	Roach
Subtask 5.2: Compare gene expression profiling in the lungs during disease development.	16-28	Xia	Roach
Subtask 5.3: Analysis of mRNA gene expression data. mRNA data will be analyzed using Affymetrix Expression Console® and BioConductor package.	16-28	Xia	Roach
Subtask 5.4: Correlation of metabolomics profiling to gene expression profiling of lung tissues in two independent models of lung damage.	16-28	Xia	Roach
Milestone: *Comprehensive metabolomics profiling of erythrocytes coupled with integrated systems biology analysis of pulmonary gene expression to identify molecular basis underlying hypoxemia-induced lung damage and disease progression. Discuss results.*		Xia	Roach
Specific Aim 2: Conduct preclinical studies to determine the therapeutic effects of in hypoxia-induced lung damage and disease progression by inducing circulating adenosine, erythrocyte 2,3-BPG and O_2 release.			
Major Task 6: To Use wild type (WT) mice to test if metformin reduces hypoxia-induced lung damage and disease progression.		Xia	
Subtask 6.1: To assess the preventive and treatment effects of dipyridamole on hypoxia-induced lung damage model	28-32		
Major Task 7: Conduct preclinical studies to determine if dipyridamole can reduce hypoxemia-induced lung damage and conduct genetic studies to identify molecular basis underlying beneficial role of erythrocyte ADORA2B signaling in hypoxemia-induced lung damage and disease progression.			
Subtask 7.1: To assess the preventive and treatment effects of dipyridamole on bleomycin-induced lung damage model.	32-36		
Milestone: *Provide preclinical evidence of therapeutic effects of dipyridamole to treat and prevent hypoxemia-induced lung injury in two independent two models of lung damage. Discuss results.*		Xia	Roach

Specific Aim 3: To use metformin and dipyridamole to elevate oxygen delivery in healthy humans experiencing hypoxia to examine the consequences on physical and cognitive performance, and to choose the most effective approach to manipulating oxygen delivery for future studies on COPD patients.			
Major Task 8: Obtain Regulatory Approvals (IRB)			Roach
Subtask 8.1: Obtain University of Colorado and Alma College Institutional Review Board (IRB) approval for study.	1-6		
Subtask 8.1: Obtain Department of Defense (DoD) Human Research Protection Office (HRPO) approval for study. *Outcome: human studies protocol approval Univ of Colorado, Alma & DOD.*	6-12		
Major Task 9: Prepare for and conduct field studies			Roach
Subtask 9.1: Begin recruiting 100 sea level volunteers and screening for inclusion/exclusion criteria. a) Each potential volunteer will complete physical examination to assess compliance with inclusion and exclusion criteria; each potential volunteer will complete the Army physical fitness test (APFT) with a minimum of 60 points based on each of the three age adjusted APFT criteria for push ups, sit-ups, and 2-mile run.	12-30		
Subtask 9.2: Continue recruiting 100 sea level volunteers and screening for inclusion/exclusion criteria.	12-30		
Subtask 9.3: Identify top 70 volunteers according to physical fitness test scores for further screening. a) Choose 60 from the 70 selected for matching according to sex, height, weight, and oxygen consumption from 3 groups of 20 subjects who have similar sea level characteristics; b) Conduct repeated cognitive function and physical fitness testing on 60 to ensure performance stability.	12-30		
Subtask 9.4: Conduct practice test weekend with sea level staff in Colorado to standardize testing procedures.	12-30		
Subtask 9.5: Schedule volunteers for weekend trips to Colorado and conduct serial weekend testing with 8-12 subjects per group.	12-30		
Subtask 9.6: Conduct daily data entry of physical performance, oxygen transport and cognitive function scores.	12-30		
Milestone: *Obtain complete data sets on 60 subjects. Discuss preliminary results.*		Xia	Roach
Major Task 10: Finalize field studies and analyze data			Roach
Subtask 10.1: Complete any additional field studies needed to meet goal of 20 subjects in a group, 60 subjects total.	24-36		
Subtask 10.2: Break drug code and begin to analyze data to determine effects on physical performance, O_2 transport and cognitive function at high altitude.	24-36		
Subtask 10.3: Continue with quarterly performance reporting.	24-36		
Subtask 10.4: Complete data analysis, publish results in suitable journal, and complete final technical report.	24-36		
Milestone: *Demonstrate effectiveness of metformin and dipyridamole to improve tissue O_2 delivery in hypoxic humans. Discuss results.*	24-36	Xia	Roach
Major Task 11. Joint creation, editing and approval of final report for scientific, regulatory and financial obligations for this proposal.	24-36	Xia	Roach

Attachment 6. Impact Statement

Our project has the potential to transform treatment and prevention of respiratory and cardiovascular diseases with a component of hypoxia. In all such diseases the delivery of oxygen to all tissues of the body is limited, and this in turn limits physical and cognitive performance. In the **short-term** as a direct consequence of this study we will learn if this approach of using metformin and dipyridamole for raising tissue oxygen delivery is effective in treating and preventing two specific types of lung injury that are accompanied by hypoxia in mouse models. We will also learn if we can reverse the detrimental effects of hypoxia on otherwise healthy young men and women exercising and living for a few days in the hypoxic environment of high altitude. If the animal studies are promising, and the drugs to raise tissue oxygen delivery are effective in healthy humans, then the **long-term impact** will be to try these new ideas using metformin and/or dipyridamole to boost tissue oxygen delivery in patients with mild respiratory diseases and test for improvement in physical and cognitive performance.

The proposed studies are extremely innovative since the functional role of excess adenosine in signaling oxygen release from hemoglobin in erythrocytes had not been previously recognized. **The impact of our proposed research is highly significant since it is likely to provoke a paradigm shift in our understanding of the molecular mechanisms of improving oxygen delivery in any condition involving systemic hypoxia.** The new theoretical framework created by our research will likely reveal novel therapeutic targets for disease prevention and therapy. In particular, if our preclinical studies and pilot human studies show a beneficial role of metformin and dipyridamole in hypoxia as expected, transition to future clinical trials in COPD or other hypoxic respiratory diseases should be rapid since metformin and dipyridamole are FDA approved drugs.

In summary, the impact on patients will be substantial. Improving tissue oxygen delivery will reverse the effects of hypoxia, thus immediately improving health and overall well being. A large impact is also expected for soldiers living and working at high altitudes.

Attachment 7. Military Relevance Statement

The overall military relevance is very high. This is a proposal offering a unique approach to countering hypoxia to improve health and well-being among patients experiencing hypoxia secondary to respiratory and cardiovascular diseases, and for soldiers living and working in hypoxia at high altitudes. Hypoxia is a major component of chronic obstructive pulmonary disease (COPD), a clinically devastating disease of increasing prevalence, occurring more in military veterans than in the general population.[1-3] And many COPD patients, even those with mild disease, regularly suffer additional hypoxia during sleep.[4] Healthy troops can also be exposed to hypoxia through deployment to high altitudes (e.g. to mountainous regions of Afghanistan or in CONUS at Fort Carson, Colorado with >6000 ft average altitude of residence). Thus, for DoD personnel, veterans, and patients with COPD, tissue O_2 delivery is limited. Limitations in tissue O_2 delivery decrease overall well-being and cognitive function, and directly causes poor physical performance (even walking ability is limited).[5]

Two currently FDA-approved drugs, metformin and dipyridamole, could be effective for elevating tissue oxygen delivery in animals models of respiratory disease and in hypoxic but otherwise healthy humans. If that outcome is realized, we would immediately apply for funding to test this approach in Veterans and other patients with mild COPD as a first patient population. We would also expand our understanding of effectiveness of this approach to trials in healthy but hypoxic humans at higher altitudes and/or for longer duration exposures to hypoxia. With no regulatory hurdles to applying these drugs in a wide variety of Veteran, patient and soldier groups the idea central to this proposal has potential to provide a paradigm shift in medical care for a wide variety of groups of major importance to the DoD.

This translational proposal uses animal models to test treatment and prevention of hypoxia-induced respiratory diseases, and a human model to test improving tissue oxygen delivery for rapid translation to patient groups on successful completion of this proposal. We have proven through extensive prior DoD human trials that we can recruit sea level volunteers with a fitness and demographic distribution similar to moderate-to-highly fit soldiers. We are aware of no reason to assume these young civilian recruits do not mimic for physiological studies their counterparts in the military. Using civilians allows us to complete these groundbreaking studies in a timely manner. If we proceed from these studies to experiments with patients, our intent is to collaborate with local Colorado Veteran’s Affairs Hospital specialists on COPD as scientific and clinical partners on the future studies.

Thus, the proposed studies have the potential to have a major impact on important populations of concern to the DoD, from VA patients ill with COPD to warfighters deployed to high altitudes.

Attachment 8. Partnership Statement

Describe the expertise of the Initiating and Partnering PI, and how each will bring different strengths to the proposed project. Describe how the collaboration will better address the research and why the work should be done together rather than through separate efforts. Outline the contribution and time commitment of each partner, and how each will have equal intellectual input on the design, conduct, and analysis of the project. Describe how the PIs will manage the collaboration and workflow to optimize research efforts.

In this Partnering PI grant proposal, Aims I and II are proposed by the Xia team from Houston, and Aim III is proposed by the Roach team from Denver. Dr. Xia's team was the first to discover that circulating adenosine causes an elevation in 2,3BPG levels and thus boosts oxygen delivery at the tissue. Extending this finding, Dr. Xia and Dr. Roach combined their efforts to further discover that hypoxia-induced adenosine is likely beneficial to trigger oxygen release to enhance human adjustment to hypoxia. Thus, the partnering PIs, with multidisciplinary and interdisciplinary expertise, have already made significant contributions to understanding how to improve O2 delivery in hypoxemic humans, whether the root cause is respiratory disease or other environmental causes of hypoxemia. Dr. Xia's team has expertise in the genetic, molecular and cellular aspects of adenosine and 2,3-BPG metabolism in animal models of hypoxia and respiratory disease. In those animal models her team can test treatment and prevention of respiratory disease in a way that would not be possible in human studies. One of the strengths of this collaboration is that new discoveries of additional modulators of oxygen binding and tissue oxygen delivery can be discussed and translated rapidly to human experiments, as we have already shown we can do with the combined mouse and human studies leading up to this proposal.

Dr. Roach's team has expertise in integrative human physiology as shown by the DoD-funded experiments that revealed an important role for elevated adenosine levels for positive human adjustment to hypoxia. With their expertise in human physiology spanning the collection and interpretation of omics data and physiological data, Dr. Roach's team brings translational medicine to Dr. Xia's mechanistic and discovery-based animal models. Another strength of this collaboration is to add integrative physiology interventions and experiments to what Dr. Xia is already doing with her animal studies. This will include exploring exercise interventions, pulmonary gas exchange and pressure measurements and other physiological perspectives that can help accelerate translation from these and future mouse experiments to humans.

The partner PIs will meet once a year at the mandated DoD conference, and once additionally in person, to discuss planning, progress and future directions. The additional in person meeting will be paid for extramural funds form Dr. Roach's lab. In addition, Dr. Xia and Roach will talk every 2 to 4 weeks by Skype as progress and workload demand to truly partner in the management and understanding of the progress being made on Aims 1-3. Each will have equal intellectual input on the design, conduct, and analysis of the project. We, Dr. Xia and Roach, are the leaders of this proposal and will put in the budgeted amount of effort and more if needed to make sure these experiments are a success. We think this design will facilitate give and take between our to two teams to make the most of the planned synergy. In our minds the synergy is circular, not just from animal models to human experiments, but also back to new experiments in animal models based on observations in human models.

It is our hope that within the next 3 years, our proposed studies will provide us new insight into the pathogenesis of COPD and we will accumulate additional evidence to proceed with enhancing O2 delivery to reduce hypoxemia for the treatment of hypoxemia secondary to respiratory disease in patients and any other humans experiencing hypoxia.